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A TREE CLASSIFICATION FOR THE SELECTION FORESTS OF THE SIERRA NEVADA[1]

By DUNCAN DUNNING

Silviculturist, Forest Service, United States Department of Agriculture

INTRODUCTION

Individuality in man is accepted without question. In domestic animals, also, good and bad individuals are generally recognized. Even in some cultivated plants—orange trees and rubber trees—the poor producers are searched out and eliminated. Indeed, individual variability is a normal condition in all groups of organisms. Yet forest trees are rarely thought of in terms of the individual. Forest products are seldom of sufficient value to justify tending the individual tree. But there is no more reason why two western yellow pine trees should grow with equal rapidity or bear equal amounts of seed because they grow under identical conditions than that two men should attain equal strength or equal mentality because they receive the same food. When to inherent variability are added the effects of a wide range of interrelated environmental factors, the great differences in the behavior of individual trees can be readily appreciated. It is adjudged a common fault to lose sight of the forest through confusion of the trees. Much more frequently in forestry the mass effect is the more obvious, and there is failure to see in their proper relationships the elementary components of the forest—the individual trees.

EXISTING TREE CLASSIFICATIONS

Thus far, within the species, foresters in the United States have necessarily been limited in the classification of trees to only slightly less generalized groups of individuals defined by differences in vigor or value. Dominant, codominant, intermediate, and suppressed, or some such classes based upon position in the crown canopy, are universally recognized. In European countries, where forestry has become most intensive, the necessity for distinguishing more clearly these differences in vigor and value has led several foresters to consider not only position in the crown canopy but crown development and stem form as well.

In Germany in 1884 G. Kraft formulated a tree classification based on crown development (*6*).[2] This was amplified in 1897 by C. R. Heck (*4*), who distinguished stem classes within each crown class. Marked differences in growth (*5*) and seed bearing (*14*) between these tree classes have been clearly determined from the study of sample plots over long periods.

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[1] Reference is made by number (italic) to "Literature cited," p. 770.

In 1902 German forest research institutions agreed upon a tree classification to be used in thinning research (*9, p. 200*). In Switzerland, France, Denmark, Finland, and Sweden the question of tree classes is also dealt with extensively in the literature of thinning. The late Gunnar Schotte in the publication of the Swedish State Forest Experiment Station in 1912 gave a review of tree classifications hitherto in use, and added one of his own based partly upon position in the crown canopy, partly upon crown development, and to a lesser degree upon stem form (*12, 8*).

THE NEED FOR A NEW CLASSIFICATION

All these classifications, so far as known, apply to even-aged stands, for the most part well stocked, and of comparatively simple composition. In the pine stands of California the situation is complicated by irregularity in age, understocking, and mixtures of several tolerant and intolerant species. In addition to position in the crown canopy, crown development, and stem form, the age of each tree must be considered in any grouping based on capacity for growth and seed production. The conventional crown classification applicable to even-aged stands, if recognized at all, is an unsatisfactory index of vigor under these conditions. In the western yellow pine stands of the Southwest some improvement has been effected by the recognition of two broad age classes—"blackjacks" and "yellow pines" (*7*). In California a rough segregation of age classes is now practiced in marking, but is recognized to be inadequate. Selection by diameter limits is a poor makeshift, to be used only when marking is not feasible.

The history of older Forest Service cuttings and examinations of marking on several of the most important recent sales indicate clearly that there is still lack of reasonable uniformity in the application of the same marking principles in similar stands. At the same time there is a lack of adaptability in applying the principles to varying conditions. Incorrect marking has frequently resulted in rates of growth much below the capacity of the site. Far too many unproductive trees are being retained.

The policy of the Forest Service in the California pine region is to reserve 20 to 30 per cent of the original stand in sound thrifty trees capable of good growth and likely to survive windfall, insect attacks, or fire, to make feasible a second cutting in comparatively inaccessible areas in reasonable time. A considerable portion of this reserve must be high-quality timber, and this necessarily means retaining rather large trees. As a source of seed in case the advance reproduction should be destroyed by fire, four or more seed trees per acre, also of rather large size, must be left. Far more skill is demanded in marking for a large reserve than for a heavy selection or seed-tree cutting. On many cuttings the provision for reservation of a certain percentage of the stand has been too strictly followed, with insufficient consideration for the condition of the stand or site variations. A recognized system of tree classification would no doubt result in uniformly better marking. As a basis for comparison of marking jobs in sales inspections such a system has obvious advantages.

Forest entomologists have demonstrated that the western pine beetle (*Dendroctonus brevicomis*) has a definite tendency to select

certain trees in endemic infestation.[3] A clear definition of the susceptible types of trees would permit their elimination by marking, and thus greatly reduce this important source of loss.

In studies of sample plots in selection stands where records are made of individual trees, a uniform system of tree classification is needed to simplify recording and permit accurate comparison of one area with another. E. J. Hanzlik has suggested using the Swedish system developed by Schotte for such work (*3*). For sample plots in even-aged stands this system doubtless works well, but omission of the age factor makes it unsuitable for application in selection stands or in cut-over areas.

A workable tree classification also offers interesting possibilities in appraisals, in marking to maintain certain standards of growth, in predicting future yields, in studies of susceptibility to fire damage, and in many other ways in which simple crown classes are now used in even-aged forests.

Whatever system of tree grouping is used in marking, it is not to be expected that there will be perfect agreement between different men. Border-line trees will be encountered in this work, just as they are in applying the accepted classifications for even-aged stands. Adherence, however, to a definite system of appraising each tree, based on easily discernible characteristics, will prevent obvious mistakes in marking, and so will raise the average rate of growth in cut-over stands, decrease losses, and improve the quality of seed trees. Agreement on a well-defined terminology is essential to mutual understanding.

BASIS FOR CLASSIFICATION

The conclusions presented here are the results of 15 years' observation of more than 20,000 numbered trees in 25 permanent sample plots covering about 300 acres, established on typical sale areas in the Sierra. Detailed crown and bole descriptions permit segregation of the trees into the classes to be described below. On this basis comparisons of growth and seed bearing have been made from measurements taken at five-year intervals. For the sake of brevity, the results for only one species, western yellow pine (*Pinus ponderosa*), are given. This species occurs on all the plots in numbers sufficient to give a reasonably good basis of data. It is widely known, and is more easily grouped into the proposed classes than any of the other species.

The proposed classification represents an effort to segregate into groups the trees with certain combinations of characteristics that are known from previous studies (*1*, *2*) to have similar influences on growth or seed bearing. It is obviously impossible to consider each of the interrelated variable elements singly. The significance of any one factor can not be isolated. Practice demands that the number of classes be small and that the factors on which they are based be readily distinguishable. In actual field tests of this system no serious difficulties have been encountered by the men, even though they had little previous knowledge of the grouping.

The major factors considered in the make-up of these classes are:

1. Four general age groups—young (less than 50 years), thrifty mature (50 to 150 years), mature (150 to 300 years), and overmature (over 300 years).

[3] Carefully controlled experiments have recently been conducted by H. L. Person at North Fork, Calif., on behalf of the Bureau of Entomology. A report of these studies is in preparation for publication in the Journal of Forestry.

2. Degree of dominance within these age groups, expressed in terms of the conventional crown classes—isolated, dominant, codominant, intermediate, and suppressed.

3. Crown development.

4. A supplementary estimate of thrift designated in three degrees of vigor—good, moderate, and poor.

The estimate of vigor is based on apparent age, degree of dominance, crown development, and, in addition, the density and color of the foliage, the form of the top (whether pointed, round, or flat), the size attained in relation to age, the color, thickness, and texture of the bark, and freedom from disease.

In marking, only sound, well-formed trees need be considered in such a classification. It is deemed undesirable to introduce complications in the form of subclasses for the multitude of defects which may possibly occur. The question of merchantability assumes priority and should be considered separately on the basis of already well-established criteria. Trees that are malformed, injured, or diseased should be removed from the stand wherever possible, and it is unnecessary to go further in segregating them by thrift classes.

In working up this material an effort was made to determine to what extent mechanical injuries and defects, such as fire scars and logging scars on the stem, fire or logging damage to the crown, broken or dead tops, etc., affect growth. The difficulty of isolating the effects of a given class of defects is apparent. On cut-over areas the number of defective trees has naturally been reduced, so that after division into comparable groups there is insufficient material to be of much significance. Damage to the crown that materially reduces the leaf area is usually reflected in a reduction in the rate of growth. In the present data no consistent relation is discernible between mechanical injuries to the stem and deficient vigor. Such injuries, when of sufficient extent to affect growth materially, should influence marking through predisposition to bring about losses and because of their effect on merchantability, rather than through their effect on growth. For purposes of study it is a simple matter to supplement the classification for sound trees with a description of defects that will permit elimination of uncertain influences from the data.

DESCRIPTION OF CLASSES

Seven tree classes are proposed, as follows (see fig. 1).

Class 1: *Age class*, young or thrifty mature; *position*, isolated or dominant (rarely codominant); *crown length*, 65 per cent or more of the total height; *crown width*, average or wider; *form of top*, pointed; *vigor*, good.

Trees of this class are rarely over 30 inches in diameter even on good sites. The bark is dark brown and roughly fissured into ridges or small plates. The foliage is rich green in color and dense, owing to retention of the needles of three to five seasons or more, except at the base of the crown. The needles are often long and coarse, especially near the top. Terminal buds are large. The top is pointed, owing to the rapid elongation of the terminal. Thrifty open-grown young trees belonging to this class are, however, sometimes round topped because of excessive lateral growth of branches near the top. On the other hand, slow-growing trees sometimes have pointed tops, due to weak development of laterals. The annual whorls of branches

and internodes are still distinct, except in the lower crown. Branches are horizontal or upward curving, except at the base of the crown where suppression is taking place. Numerous stubs of dead branches are likely to be present below the crown.

Class 2: *Age class*, young or thrifty mature; *position*, usually codominant (rarely isolated or dominant); *crown length*, less than 65 per cent of the total height; *crown width*, average or narrower; *form of top*, pointed; *vigor*, good or moderate.

Such trees are usually less than 24 inches in diameter. They are commonly the inside codominant trees of groups. The crowns are smaller and less dense than in trees of the first type. Otherwise they are similar to those of class 1.

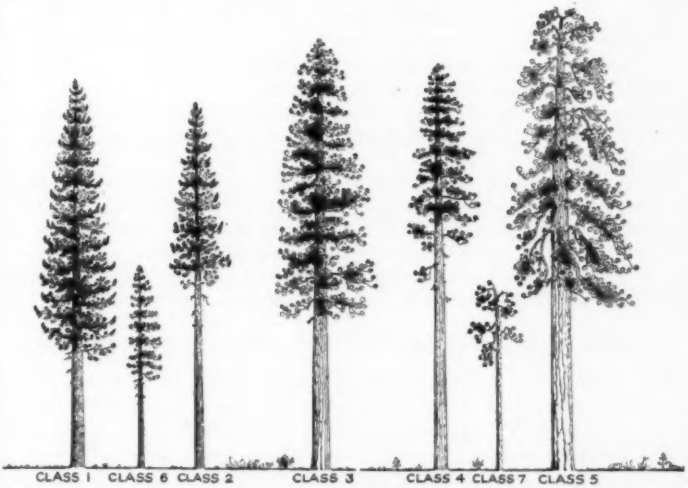

FIG. 1.—Classes 1, 2, and 6 represent young or thrifty mature trees; classes 3 and 4 mature trees; classes 5 and 7, mature or overmature trees. Form of top, crown width and length, and position in the crown cover are other determining characteristics

Class 3: *Age class*, mature; *position*, isolated or dominant (rarely codominant); *crown length*, 65 per cent or more of total height; *crown width*, average or wider; *form of top*, round; *vigor*, moderate.

These trees are ordinarily between 18 and 40 inches in diameter, depending on site quality. The bark is light brown or yellow, with moderately large smooth plates. The foliage is less dense than in class 1 trees. The top is round, because of slow height growth. The nodes are indistinct, because of incomplete whorls of branches. The branches are nearly all horizontal or drooping.

Class 4: *Age class*, mature; *position*, usually codominant (rarely isolated or dominant); *crown length*, less than 65 per cent of the total height; *crown width*, average or narrower; *form of top*, round; *vigor*, moderate or poor.

These are commonly the inside or codominant trees of this age class. Except for their small poorly developed crowns and smaller size, they are similar to class 3 trees.

Class 5: *Age class*, overmature; *position*, isolated or dominant (rarely codominant); *crown* of any size; *form of top*, flat; *vigor*, poor.

These are usually the largest trees in the stand. The bark is light yellow in color, the plates often very wide, long, and smooth, especially near the base. The bark may be thin, having weathered more rapidly than it has grown. The foliage is usually rather pale green and very thin. The needles are fairly short, appearing as tufts on the ends of the twigs. The needles of two or three seasons only may be retained, even near the top. The top is flat, the terminal rarely discernible. There is no appreciable elongation of the main axis. Scarcely any nodes are distinguishable. Nearly all the branches are drooping, gnarled, and crooked.

Class 6: *Age class*, young or thrifty mature; *position*, intermediate or suppressed; *crown* of any size, usually small; *form of top*, round or pointed; *vigor*, moderate or poor.

These are understory trees, rarely over 12 or 14 inches in diameter. The bark is dark and rough. The top is round or pointed, showing that some height growth is taking place. Whorls of branches are evident, though the internodes are short.

Class 7: *Age class*, mature or overmature; *position*, intermediate or suppressed; *crown* of any size, usually small; *form of top*, flat; *vigor*, poor.

These understory trees are rarely over 18 inches in diameter. The bark is light in color, thin, and smooth. The top is flat, the terminal rarely distinguishable. The foliage is excessively thin. The few branches present are gnarled and drooping.

The similarities and differences between these groups are perhaps more evident in the abbreviated comparison given in Table 1.

RELATIVE IMPORTANCE OF CLASSES IN THE STAND

The relative importance of the tree classes in the original and remaining stand and the proportion of the cut supplied by each are shown in Table 2, which summarizes the data from a typical stand in the Stanislaus National Forest cut over in 1923. Marking was carefully done by a marking board to conform as nearly as possible to existing cutting policy.

TABLE 1.—*Summary of tree classification by system proposed* [a]

	Age class	Crown class (position)	Crown length	Crown width	Form of top	Vigor
			Per cent			
Class 1	Y—TM	X—D (C)	65+	M—W	∧	V
Class 2	Y—TM	C (X—D)	65−	M—N	∧	V—M
Class 3	M	X—D (C)	65+	M—W	⌒	M
Class 4	M	C (X—D)	65−	M—N	⌒	M—P
Class 5	OM	X—D (C)	All.	All.	⌐	P
Class 6	Y—TM	I—S	All.	All.	⌒ (∧)	M—P
Class 7	M—OM	I—S	All.	All.	⌐	P

[a] Significance of symbols is as follows:
Age class: Y—TM=young or thrifty mature; M=mature; OM=overmature.
Crown class: X=isolated; D=dominant; C=codominant; I=intermediate; S=suppressed.
Crown width: M=average; W=wider than average; N=narrower than average; All=any size.
Vigor: V=good; M=moderate; P=poor.

TABLE 2.—*Volume and number of trees per acre and percentage of total volume in each tree class in original stand, in portion marked for cutting, and in reserved portion* [a]

VOLUME PER ACRE

Tree class	Original stand		Marked for cutting		Reserved		Percentage of each class marked
	Board feet	*Per cent*	*Board feet*	*Per cent*	*Board feet*	*Per cent*	*Per cent*
Class 1	12,772	16.6	4,373	6.9	8,399	62.8	34.2
Class 2	2,259	2.9	353	.6	1,906	14.2	15.6
Class 3	21,297	27.8	18,407	29.1	2,890	21.6	86.4
Class 4	1,708	2.2	1,708	2.7			100.0
Class 5	37,875	49.4	37,875	59.7			100.0
Class 6	211	.3	51	.1	160	1.2	24.2
Class 7	595	.8	575	.9	20	.2	96.6
Total	76,717	100.0	63,342	100.0	13,375	100.0	82.6

NUMBER OF TREES PER ACRE [b]

Tree class	Original stand		Marked for cutting		Reserved		Percentage of each class marked
	Number	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	*Per cent*
Class 1	34.2	34.4	3.4	15.9	30.8	39.5	9.9
Class 2	7.6	7.7	.3	1.4	7.3	9.4	3.9
Class 3	7.3	7.3	6.4	29.9	.9	1.2	87.7
Class 4	.5	.5	.5	2.3			100.0
Class 5	8.2	8.3	8.2	38.4			100.0
Class 6	37.4	37.6	.2	.9	37.2	47.6	.5
Class 7	4.2	4.2	2.4	11.2	1.8	2.3	57.1
Total	99.4	100.0	21.4	100.0	78.0	100.0	21.5

[a] Board-foot figures give stands per acre for trees 12 inches diameter breast high and over, Scribner Decimal "C" rule. Stanislaus plot 5, cut in 1923.

[b] Figures show number of trees per acre 4 inches diameter breast high and over.

Classes 1, 3, and 5, the large-crowned dominant trees in the three general age groups, here form 93.8 per cent of the original board-foot volume for trees 12 inches in diameter and larger. Classes 3 and 5, mature and overmature dominants, provided 88.9 per cent of the cut. These tree classes contain the highest grade material (*11*), and probably represent more than 95 per cent of the present value of the entire stand. The class 1 trees, immature dominants, which formed 16.6 per cent of the original stand, supplied but 6.9 per cent of the volume cut. Such trees were marked only when it was necessary to thin groups of trees, to facilitate logging, or when they had been badly injured by removal of other trees. Classes 1 and 2 make up 77.1 per cent of the 13,375 board feet per acre reserved, representing very little present value, since they contain comparatively little high-grade lumber. Class 3 trees represent 21.6 per cent of the reserve volume, providing a few larger seed trees and some high-grade material for a second operation.

The small-crowned dominant and codominant trees of classes 2 and 4 and the intermediate and suppressed trees of classes 6 and 7 never form more than a small part of the merchantable volume. In number of trees, however, they are often relatively important. They are of primary interest because of their influence on future yields. The numerical importance of the tree classes in the above stand is

also shown in Table 2, with the inclusion of unmerchantable trees between 4 and 12 inches. In this stand, where there was an average of 99.4 trees per acre, most of the trees were in classes 1 and 6—34.2 per cent and 37.4 per cent, respectively. Classes 2, 3, and 5 were nearly equally represented by 7 to 8 per cent of the total. There were relatively few trees in classes 4 and 7.

COMPARISONS OF GROWTH

For the sake of simplicity all the following growth comparisons are in terms of basal area, expressed as average annual rates per cent for the 15-year period. In terms of volume growth the differences shown would be accentuated, since the mature and overmature trees make practically no height growth. The size grouping is based on the diameters at the beginning of the period.

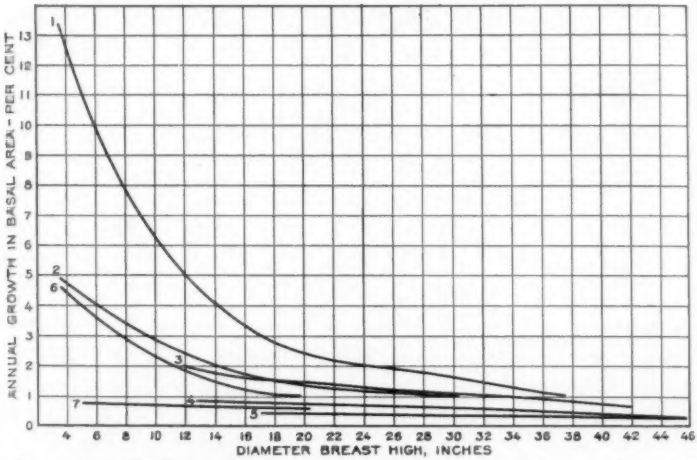

FIG. 2.—Annual basal area growth per cent, by tree classes, western yellow pine, site I (1,183 trees, 1910–1925)

Figure 2 permits comparison of the relative rates of growth of the seven tree types by diameter classes. The graph is based on 1,183 trees remaining on a first-quality site that was cut over in 1910, in the Stanislaus National Forest.

Class 1 trees are superior to the others for all diameters, but especially so in the smaller sizes. Class 2 trees have grown considerably more slowly than those of the first group, but in the smaller size classes are considerably above the trees in the remaining groups. Above 18 inches class 2 and 3 trees have grown at about the same rate, the larger crown area of the class 3 trees offsetting their greater age. The rate for class 3 shows little variation with size.

The trees of classes 4, 5, and 7 show still less variation with diameter, growing at a hopelessly slow rate for all sizes. The younger trees of class 6 give some promise, and the fact that few of them can be cut under present economic conditions is not a serious matter.

It is evident that a good rate of growth can not be expected from mature and overmature trees of classes 3, 4, 5, and 7, regardless of diameter, even on the best sites. Above 28 or 30 inches, differences

in rate of growth between the seven groups become unimportant. For these larger trees the risk of loss and seed-bearing ability should govern the choice in marking.

It is apparent from Figure 2 that age is the most important factor to be considered. Crown development and crown class differences are far more important in the young and submature than in the mature and overmature age classes.

A summary of the growth produced by this same stand is given in Table 3. Class 1 trees, which represented but 29.4 per cent of the residual stand, produced 57.7 per cent of the total growth and maintained the highest annual rate, 3.05 per cent. Class 2 trees grew only half as rapidly. Class 3 trees have not justified their retention from the standpoint of growth, and yet the annual rate of nearly 1 per cent is fairly good, and increase in value justifies reserving a considerable proportion of such trees. Little can be said in defense of the reservation of nearly one-fourth of the stand in classes 4 and 5.

TABLE 3.—*Percentage of total basal area in 1910 and total growth 1910–1925 represented by each tree class, with annual growth rate (volume) maintained by each class over the 15-year period* [a]

Tree class	Total basal area, 1910	Growth produced, 1910–1925	Annual growth rate
	Per cent	*Per cent*	*Per cent*
Class 1	29.4	57.7	3.05
Class 2	11.4	11.2	1.53
Class 3	27.9	17.8	.98
Class 4	5.1	1.9	.59
Class 5	18.8	4.3	.35
Class 6	3.7	5.6	2.34
Class 7	3.7	1.5	.65
Total	100.0	100.0	1.56

[a] Western yellow pine, Stanislaus plots 2, 3, and 4. Site I. Cut over in 1910.

A similar relationship is shown graphically for a much larger area in Figure 3.

It should be remembered that the foregoing growth comparisons include the acceleration due to release by cutting, which should have culminated within 15 years after the thinning. The trees which were subordinate in the original stand have not generally improved sufficiently to equal the rates maintained by former dominants. It is usual for higher classes to decline to subordinate classes, but extremely exceptional for the reverse process to occur. Acceleration of growth is a minor consideration in the prevailing type of cutting with its tendency toward grouping of reserves. It is better to reserve trees already dominant than to rely upon the enhancement of increment in understory trees. This is particularly true in stands where the merchantable trees are all comparatively old. This point should be carefully considered in thinning groups.

COMPARISONS OF LOSSES

The ultimate success of marking is dependent upon both potential growth rates, as discussed above, and survival of the growing stock.

On the plots from which the data in Table 4 were taken there were 4,669 living trees, 4 inches in diameter and over, after cutting in 1910.

Between 1910 and 1925, 172 trees died. The distribution by classes of the total trees in 1910 and of the trees which died is shown in number of trees and basal area in Table 4. The fourth and last columns of figures show the relative liability of the different classes to loss, or the ratio of occurrence in the losses to occurrence in the stand.

On a numerical basis it is evident that the class 1 trees are the lowest risk. They are represented in the losses only about one-seventh as frequently as in the total stand. The liability to loss is from five to fifteen times greater for the other classes. Class 7 has the highest risk factor, followed by classes 4, 2, 6, 5, and 3. It is fortunate for the reserve policy that the class 3 trees have the lowest risk factor of any class except the first.

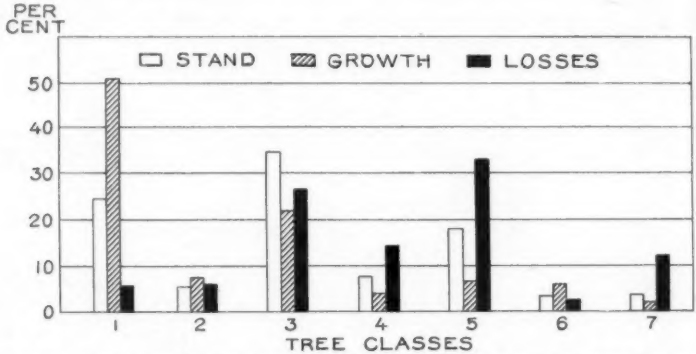

FIG. 3.—Apportionment between the seven tree classes of the total basal area of the stand, the total growth produced between 1910 and 1925, and the total loss in the same period. The data are taken from 4,669 trees totaling 7,855.64 square feet in basal area

TABLE 4.—*Apportionment of stand and mortality in each tree class, 1910–1925, in percentage of number of trees and basal area, with relative liability to loss of the trees in each class* [a]

Tree class	Apportionment in trees			Apportionment in basal area		
	Total stand	Mortality	Ratio mortality to stand [b]	Total stand	Mortality	Ratio mortality to stand [b]
	Per cent	*Per cent*		*Per cent*	*Per cent*	
Class 1	34.0	4.7	0.14	24.9	5.2	0.21
Class 2	8.4	13.4	1.60	5.7	5.6	.98
Class 3	15.4	11.6	.75	35.0	26.7	.76
Class 4	6.6	11.6	1.76	7.8	14.5	1.86
Class 5	5.5	6.4	1.16	19.5	33.6	1.72
Class 6	16.5	23.2	1.41	3.2	2.3	.72
Class 7	13.6	29.1	2.14	3.9	12.1	3.10
Total	100.0	100.0		100.0	100.0	

[a] Based on 4,669 trees having a total basal area in 1910 of 7,855.64 square feet. Mortality was 172 trees, totaling 334.99 square feet in 1910.

[b] Loss ratios, or risk factors, were obtained by dividing the figures in column 3 by those in column 2 and those in column 6 by those in column 5, class by class.

The significance of the loss data is more clearly brought out through basal area comparisons. On this basis the class 1 trees still represent much the lowest risk, followed by classes 6, 3, 2, 5, 4, and 7. The

greatest actual losses occurred in classes 3, 4, and 5, partly because the trees in these groups were large and formed a considerable portion of the reserve and partly because of a higher loss rate, especially in classes 4 and 5, as indicated by the high ratios in the last column of Table 4. (See also fig. 3.) If the relative risks were expressed in terms of value exposed to loss rather than basal area, the disparity would be much greater, since classes 3, 4, and 5 produce the highest percentage of upper-grade lumber.

The greatest single cause of mortality was bark beetles (Dendroctonus), which killed 61, or 35 per cent, of the 172 trees and accounted for 50 per cent of the basal-area loss. The distribution of insect losses by tree classes as compared with losses from other causes is given in Table 5.

Only a small part of the loss from insects occurred in the younger tree classes 1, 2, and 6, the greatest portion, nearly half the total, being in class 5. The relative risk of loss from insects in the various classes may be expressed by the ratios in Table 5, derived by dividing the percentage of the total basal-area loss occurring in each class by the percentage of the total basal area of the stand in each class. For comparison, similar ratios for other causes of loss are also shown.

TABLE 5.—*Distribution of losses in basal area from insects and other causes by tree classes, with relative liability to loss of the trees in each class*

Tree class	Insect damage		Other loss	
	Distribution of loss	Ratio mortality to stand	Distribution of loss	Ratio mortality to stand
	Per cent		*Per cent*	
Class 1	3.4	0.14	7.0	0.28
Class 2	5.4	.95	5.8	1.02
Class 3	14.6	.42	38.6	1.10
Class 4	16.2	2.08	12.8	1.64
Class 5	48.7	2.50	18.6	.95
Class 6	1.1	.34	3.5	1.09
Class 7	10.6	2.72	13.7	3.51
Total	100.0		100.0	

The probability of insect loss is greatest in class 7. Such trees appear 2.72 times as frequently in the losses as they do in the stand as a whole. The high factors for classes 4 and 5 are particularly significant because of the high-grade material contained in trees of these types. The above comparisons indicate that only trees of classes 4 and 5 are more subject to losses from beetles than from other causes. For class 5 trees the risk from beetles is more than two and one-half times as great as from other causes. It is worth noting that class 3 trees, which should properly make up the bulk of the better quality of timber reserved, are apparently less liable to damage from insects than from other causes. These relationships are shown graphically in Figure 4.

The above comparisons in basal area show the combined results of relative susceptibility and amount of timber exposed to loss. The selective tendency alone is more strikingly indicated by numerical comparisons, as shown graphically in Figure 4. The small-crowned mature codominants of class 4 are indicated to be the most liable to insect damage.

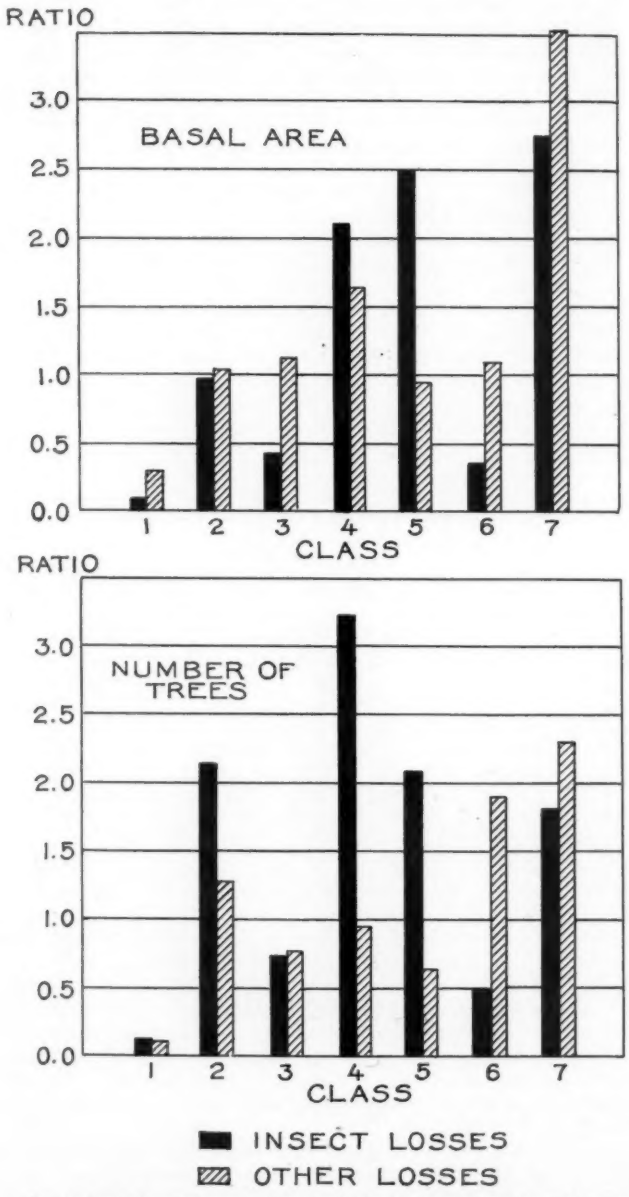

FIG. 4.—Ratio of apportionment of insect and other loss between tree classes to apportionment of stand between classes; in other words, the relative liability to loss of the different classes

The relative susceptibility of various tree types to killing by the western pine beetle, as indicated above, is in close agreement with the carefully controlled experiments by H. L. Person, of the Bureau of Entomology, already referred to. The elimination of susceptible trees in cutting would doubtless lessen endemic insect damage, the most important cause of loss on cut-over areas.

COMPARISONS OF SEED BEARING

The seed-bearing capacity of forest trees has been shown by many investigators to be affected by a large number of environmental phenomena and inherent qualities. In the present study the aim has been to determine only how well the proposed tree classes integrate seed-bearing capacity, and no attempt has been made to record the more fundamental biological influences involved. Even so, the present results are but tentative.

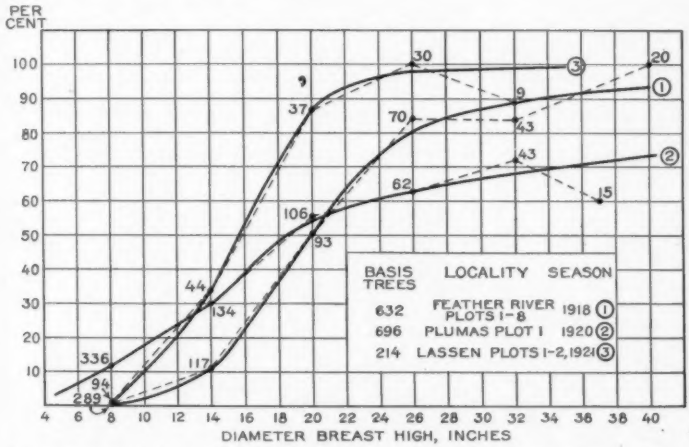

FIG. 5.—Percentage of trees of various diameters bearing cones, taken in three different localities in good average years. Data are for western yellow pine only

The physical difficulties encountered in a quantitative determination of the amount of seed borne are obvious. Unfortunately, too, there have been no really heavy general seed crops since the trees have been under observation, such as would permit comparison of seed bearing by similar tree types under different site conditions, or the consistency of bearing by the same trees from year to year. The data available necessarily limit comparisons to single localities and certain years. There are, however, certain outstanding differences which justify consideration. At present it is impossible to go further than to indicate what types of trees appear to be the best seed bearers. Determination of the exact quantity and quality of seed that is produced and remains undestroyed by insects, rodents, etc., must be left for further study.

Observation indicates that occasionally a potentially good seed bearer sets a heavy crop of fertile cones all of which are destroyed before maturity by cone beetles or rodents. For all species except

sugar pine (*Pinus lambertiana*) it has been found impossible to count the immature cones early enough to avoid these losses. In the absence of exact knowledge it is assumed for the present that such damage is not restricted to any particular tree class and that the figures presented are therefore comparable.

Under similar conditions of site, the major factors influencing seed bearing appear to be age, position in the crown canopy, and crown development. Since the size attained is closely correlated with age, crown class, and crown development, it is to be expected that there exists a close relationship between diameter and seed-bearing capacity. This relationship is shown in Figure 5 for three localities in different years.

Below 8 inches a negligible percentage of the trees bore cones. Between 8 and 26 inches the proportion of trees bearing cones increased rapidly. Above 26 inches practically all the trees bore some cones. At 20 inches, the size ordinarily regarded as representing satisfactory seed trees (*13*), from 50 to 90 per cent of the trees bore seed in these years.

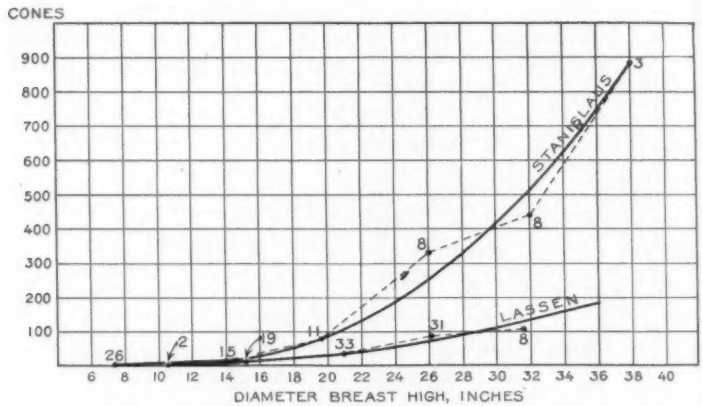

FIG. 6.—Number of cones per tree at various diameters. Count taken on western yellow pine on Lassen plots 1 and 2, and Stanislaus plot 5, 1925

It is well known that for many species there is a fairly definite alternation of seed years and barren years. Thus in the sample plots on the Lassen National Forest, only 62.5 per cent of the trees bore seed in both the seasons of 1921 and 1926. Furthermore, in the seasons of record the seed crops were never exceptional. Were records for a number of years available, covering one or more years of heavy crops, they would doubtless show much higher percentages of trees capable of bearing seed than the chart indicates.

The number of cones borne also varies consistently with diameter, which serves as an indirect integration of age and other factors. The number of cones per tree for two areas is shown in Figure 6 for the year 1926. The difference between the two curves illustrates the great local variation in the seed crop of the same season, the Lassen area being situated 160 miles north of the Stanislaus. These curves indicate that, in years of ordinary seed crops at least, rather large trees are necessary for the production of considerable quantities

of seed. Although 50 per cent or more of the 20-inch trees may be capable of bearing seed, the number of cones borne by them is relatively small.

The foregoing discussion indicates that the best seed trees will be found in the tree classes represented by the larger sizes. The summary in Table 6 of typical data from the Feather River group of plots makes this clearer.

The indicated differences are doubtless a result of the combined influences of age, crown development, and position or size. Comparison for a given size class is impossible because, in the nature of things, there is no one size class in which all the tree classes are well represented. It is apparent, however, that seed-bearing trees will most frequently be found among the principal trees of classes 1, 3, 4, and 5, and that classes 2, 6, and 7 provide an insignificant number of seed bearers. This relationship has been well established by a considerable number of investigations elsewhere (*14*, *15*).

TABLE 6.—*Percentage of trees in each class bearing cones and average diameter breast high of each class; eight Feather River plots, 1918*

Tree class	Trees recorded	Trees bearing cones	Average class d. b. h.
	Number	*Per cent*	*Inches*
Class 1	214	22.9	15.0
Class 2	84	14.3	13.5
Class 3	154	55.8	25.7
Class 4	52	40.4	20.4
Class 5	4	100.0	38.8
Class 6	97	1.0	7.3
Class 7	27	11.1	11.4
Total	632	27.9	

As to quantities of seed per tree, the scanty data from present observations and the more ample results of other investigators (*10*) leave little doubt that trees of the types found in classes 2, 6, and 7 bear but few cones. The largest numbers of cones per tree thus far counted were for trees of class 3.

SUMMARY

A tree or thrift-class grouping applicable to selection stands is needed which, unlike the conventional crown-class and other tree classifications for even-aged stands, will be suitable for all-aged mixed forests. On the basis of observations of permanent sample plots over a period of 15 years a tree grouping is proposed comprising seven classes defined by combinations of easily observed factors influencing vigor. The major factors considered are age, degree of dominance, and crown development. Confirmatory indications of relative vigor considered are form of top, color and density of foliage, character of bark, size, etc. Trees should be judged undesirable on the basis of unmerchantability and liability to loss rather than rate of growth.

Marked differences in rates of growth and susceptibility to loss demonstrate that the grouping proposed is a reliable integration of vigor.

Class 1 trees grow at the best rate and have the lowest loss liability factor. They are the least susceptible to insect attacks. They are good seed bearers when of sufficient size. The present value of timber from such trees is comparatively low. They should practically always be retained when sound.

Class 2 trees make fair growth, but are rather liable to loss and are poor seed bearers. They should be marked in preference to the larger class 1 trees when there are sufficient other thrifty trees to make up the reserve, or in thinning groups.

Class 3 trees grow rather slowly, but can be expected to increase in value without great risk of loss. They are good seed bearers. Such trees are desirable for retention as seed trees, or to constitute a moderate reserve of high-quality material for a second cutting in a reasonably short time. The usual tendency is to retain too many trees of this type. However, where sufficient class 1 trees are lacking, where good seed trees are needed, or where a second cutting must be provided for, there should be no hesitancy in leaving class 3 trees. Care should be taken not to include in this class overmature, slow-growing trees. Size varies greatly with site, but only exceptionally will trees of this type be found over 30 inches in diameter.

Classes 4 and 5 trees produce practically no growth even on the best sites. Their liability to loss is high. Their retention involves a large and insecure investment in high-quality timber. They should always be cut, unless there are no other available seed trees.

Classes 6 and 7 trees are usually too small to be merchantable. Class 6 trees grow fairly well and give promise of later development if released. They bear practically no seed. When merchantable they should be cut, unless relief from competition is insured by removal of other trees. Class 7 trees are undesirable from every standpoint and should always be cut if merchantable.

Seed trees should be of classes 1 or 3, and from 20 to 30 inches in diameter.

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AN ACANTHOCEPHALID, PLAGIORHYNCHUS FORMOSUS, FROM THE CHICKEN AND THE ROBIN [1]

By MYRNA JONES

Junior Zoologist, Zoological Division, Bureau of Animal Industry, United States Department of Agriculture

INTRODUCTION

Some acanthocephalids from the small intestine of a chicken, collected at Vineland, N. J., and through the courtesy of J. J. Black sent to the Zoological Division of the Bureau of Animal Industry, were identified as *Plagiorhynchus formosus* Van Cleave, 1918.[2] This report is of interest since, so far as the writer can ascertain, no members of the Acanthocephala have been recorded from the chicken. *P. formosus* is reported here also from another new host, the robin (*Planesticus migratorius*).

ECONOMIC IMPORTANCE

The possibility should be kept in mind that what is at first an accidental and economically unimportant transfer of a parasite of wild birds to poultry, in time may result in the parasite's adaptation to the new host and its subsequent widespread distribution. Something of this sort appears to have happened in the case of such economically important parasites as *Thysanosoma actinioides*, *Fasciola magna*, *Oesophagostomum columbianum*, and other worms. It is of interest to note that since sending in the first specimens, Black has reported finding another such acanthocephalid in a Barred Plymouth Rock cockerel.

DESCRIPTION OF SPECIMENS

The material from the chicken consisted of one male and two females, all immature. In view of this immaturity of the specimens they were considered in some detail and compared with the type material as far as possible.

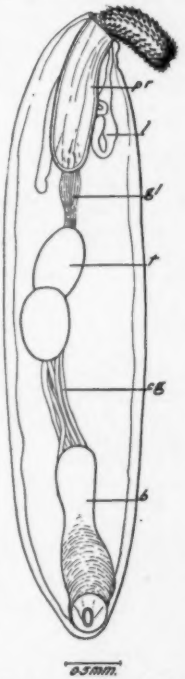

FIG. 1.—*Plagiorhynchus formosus*. Immature male from chicken: *b*, bursa; *cg*, cement glands; *gl*, genital ligament; *l*, lemniscus; *pr*, proboscis receptacle; *t*, testis

Male.—The body (fig. 1), which is without spines, is elliptical in shape; its maximum length is 5.5 mm. and its maximum diameter 1.02 mm. The cylindrical proboscis, bent at approximately a 60° angle with the body, is 0.725 mm. long and 0.325 mm. in greatest diameter, and is armed with 16 parallel, alternating rows of hooks with from 12 to 13 hooks in each row; the tip of the proboscis is slightly inverted. Beginning at the posterior end of the proboscis there is the following variation in hook lengths, in microns, for the 13 successive hooks in one longitudinal row: 40, 40, 56, 60, 64, 60, 60, 64, 60, 52, 56, 48, and 40. The double-

[1] Received for publication Mar. 22, 1928; issued July, 1928.

[2] VAN CLEAVE, H. J. THE ACANTHOCEPHALA OF NORTH AMERICAN BIRDS. Trans. Amer. Micros. Soc. 37: [19]-47, illus. 1918.

walled proboscis receptacle, attached to the base of the proboscis, is cylindrical with a rounded base, and measures 1.25 by 0.325 mm. The prominent lemnisci are 2.63 by 0.155 mm. in size. The testes are median in the body, and lie in tandem touching each other; they are of equal size, measuring 0.55 mm. long by 0.457 mm. broad. The six cement glands are long and slender, lie very close together, and extend from the posterior testis to the bursa.

Female.—The females (fig. 2) are ellipsoidal in shape and are shorter than the males. They measure 4.48 by 1.12 mm. and 3.78 by 1.28 mm. in maximum lengths and diameters. The probosces are 0.64 by 0.28 mm. and 0.78 by 0.306 mm., respectively, their tips being considerably inverted. The probosces bear 16 parallel longitudinal rows of hooks; 10 to 12 hooks in a row are to be seen clearly and 2 to 4 more per row can be made out in certain parts of the inverted tips. Beginning at the posterior ends of the probosces there are the following variations in hook lengths in microns for 12 successive hooks in a row: 40, 60, 76, 72, 68, 68, 68, 76, 78, 72, 72, and 60, in one specimen, and 48, 48, 64, 68, 72, 80, 76, 76, 76, 80, 80, and 72 in the other. The proboscis receptacles with their rounded bases measure 1.54 by 0.309 mm. and 1.54 by 0.344 mm. in the two specimens. The ovarian masses, situated medianly, are loosely lobate.

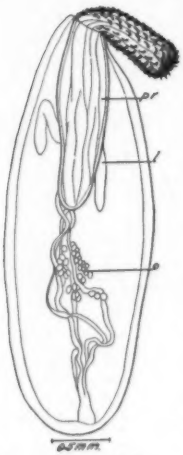

FIG. 2.—*Plagiorhynchus formosus*. Immature female from chicken: *l*, lemniscus; *o*, ovary; *pr*, proboscis receptacle

These specimens evidently belong to the genus *Plagiorhynchus* Lühe, 1911, since they possess the following diagnostic characters of that genus: Proboscis with hooks in parallel, alternating rows; body proper without spines; no subcuticular nuclei; no spherical enlargement followed by narrow, elongated neck behind proboscis; proboscis receptacle a double-walled sac attached at base of proboscis; six cement glands present.

Van Cleave (1918)[3] gives this specific description for *Plagiorhynchus formosus:*

Body about 10 mm. long, elliptical to slightly ovoid. Proboscis practically cylindrical, diameter about one-third of length; armed with 16 longitudinal rows of 13 to 14 hooks each. Cement glands long, tubular. Hard-shelled embryos inside body of female elliptical, 48μ to 60μ by 12μ to 20μ in diameter.

COMPARISON WITH TYPE MATERIAL

The specimens from the chicken differ in certain respects from the type material, particularly in the matter of size. The type male is 8.5 by 2 mm., while the male described here is but 5.5 by 1.02 mm.; the type female is 9.5 by 2 mm., as compared to 4.48 by 1.12 mm. and 3.78 by 1.28 for the females from the chicken. The probosces show somewhat less marked differences in size, particularly if it is kept in mind that the tips of the smaller specimens are much more inverted. The types measure 1.06 by 0.33 mm., while the others are 0.725 by 0.325 mm., 0.64 by 0.28 mm., and 0.78 by 0.306 mm. The hooks also show some differences in length; those of the type material vary from above, vary from 48μ to 80μ, 40μ to 78μ, and 40μ to 63μ. In all speci- 65μ to 83μ in a longitudinal row, whereas the three others, as given mens the smallest hooks are at the base of the proboscis, and the longest hooks approximately halfway toward the tip.

[3] VAN CLEAVE, H. J. Op. cit.

These quite consistent differences in size do not seem of specific significance in view of the immaturity of the material from the chicken and also its presence in an abnormal host. Since 13 hooks are observed in most of the longitudinal rows in the proboscis of the male from the chicken, and 2 to 4 hooks are made out in the inverted tip of the female proboscis in addition to the 10 to 12 clearly seen, the possibility of a difference in number of hooks per longitudinal row from the number in the type seems negligible. A distinguishing feature, the occurrence of the testes in the new material midway in the body, rather than pre-equatorial and close to the proboscis receptacle as in the type male, is regarded as probably not of specific importance considering the difference in maturity of the specimens.

The points of agreement of the material from the chicken with the specific diagnosis of the type, aside from generic characters, include the elliptical shape of the body, the cylindrical shape and armature of the proboscis, and the long, tubular, cement glands. It is believed, therefore, that the specimens described here from a chicken are *Plagiorhynchus formosus*.

This species has been reported previously from the flicker (*Colaptes auratus*) collected at Bowie, Md., and from the crow (*Corvus americanus*) collected at Washington, D. C. A new host record of particular interest here is that of the robin (*Planesticus migratorius*). Three specimens of *Plagiorhynchus formosus* were collected by Black from a robin found paralyzed on the poultry farm from which the infected chicken had come. These specimens from the robin, a female and two males, are mature and conform closely to the type material in all respects. *Gallus domesticus* is undoubtedly an accidental host. However, the record is of economic interest as affording new data in regard to the interrelationships of wild birds and poultry in connection with the transfer of parasites from one to the other.

EFFECTS OF FASTING AND THE METHOD OF PREPARATION OF FEED UPON THE DIGESTIVE PROCESS IN DAIRY CATTLE [1]

By W. B. NEVENS [2]

Department of Dairy Husbandry, University of Illinois

INTRODUCTION

Studies of the process of digestion in ruminants have contributed facts of fundamental importance to the science of animal nutrition. Investigators have studied not only the chemical reactions and transformations occurring in food substances during their passage through the alimentary canal, but also the mechanical features of the process. Researches in this field have contributed much toward a solution of the feeding problems of farm livestock.

There are still many phases of the digestive process of ruminants concerning which our knowledge is not entirely clear. Armsby [3] states:

> In the ruminant, water and liquid feeds may pass quite directly to the abomasum, although as a matter of fact, they seem to reach all four divisions of the stomach. The more bulky feeds, however, fail to pass through the esophageal canal but enter the rumen and reticulum * * *. The rumen is so large that it always contains a considerable amount of material and the new feed when swallowed is more or less completely mixed with that already in the rumen by the peristaltic action of the latter, thus tending to prolong its stay. The liquid or finely comminuted portions probably pass on directly to the omasum, or manifolds, and the abomasum, but the bulk of the feed undergoes the process of rumination.

The findings of Völtz [4] with regard to the course of liquids in the stomachs of sheep do not agree with the statements of Armsby, but they do agree with respect to solid foods. It was found in Völtz's experiments that—

> When a sheep was killed directly after drinking a large amount of water to which alcohol had been added, nearly all of the alcohol was recovered in the paunch, showing that liquids are not immediately transferred to the abomasum. The same holds for solid foods, as is shown by mixing with the food finely divided silver and finding this in the paunch.

Amadon,[5] from observations made through an artificial opening into the rumen, concludes that—

> Ground feed and other concentrates may pass directly into the honeycomb during the course of the meal and we have even observed a passage of ground feed occurring from the honeycomb to the manyplies and true stomach during the eating period. The condition which determines the route to be followed is that of weight, all light food entering the back part of the paunch while a portion of the heavy food passes directly into the honeycomb * * *. Whole

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[1] Received for publication Mar. 2, 1928; issued July, 1928.

[2] Grateful acknowledgment is made to O. R. Overman, O. F. Garrett, and A. K. Joshi, of the Division of Dairy Chemistry, and to W. J. Huck, herdsman in charge of the experimental dairy herd, for assistannce in carrying out the analytical work. The feeding, collection of material from the animals, and preparation of the samples for analysis were done by the author with the assistance of W. J. Huck.

[3] ARMSBY, H. P. THE NUTRITION OF FARM ANIMALS. p. 81-82, illus. New York, The Macmillan Company. 1917.

[4] VÖLTZ, W. UEBER DEN DIREKTEN TRANSPORT DES FUTTERS UND DES TRÄNKWASSERS DURCH DEN SCHLUCKAKT BEIM WIEDERKÄUER. Med. Klinik 7: 1296-1297. 1911. (Abstract in Expt. Sta. Rec. 29: 66, 1913.)

[5] AMADON, R. S. THE OX STOMACH. SOME FACTS WHICH CATTLE OWNERS SHOULD KNOW. N. Dak. Agr. Expt. Sta. Bul. 196, 16 p., illus. 1926.

corn and heavy metal objects such as nails therefore remain in the depths of the honeycomb. The whole corn kernels are passed on through the digestive system but there is no evidence to show that such is the case as regards the foreign bodies, nails, etc.

It might be assumed that the fullness of the stomach at the time of a meal would affect the course of the food swallowed. In order to test the correctness of this theory, some of the animals used in these experiments were fasted for several days and some were full-fed up to the time of slaughter.

The fasting experiments are of especial interest in view of the fact that Forbes [6] and his coworkers have recently published a number of papers reporting experiments in which their calculations are based upon the fasting katabolism of dry cows and steers. The authors state, however, that the method is still in process of development and has not yet been standardized. The following excerpt [7] illustrates one of the attempts of the investigators to arrive at a standard procedure for bringing animals into a condition of true fasting.

> The experimental routine in the last two experiments mentioned was characterized by one noteworthy innovation—the introduction of the idea of special treatment of the experimental subject to bring about as promptly as possible a condition of true and complete fast.
>
> For this purpose a physic was given to steer No. 260, after which roughage was withheld for one day and grain for one-half day; then the paunch was washed out by means of a stomach pump, and an enema was given. The withholding of roughage for one day seems to have permitted the paunch to clear itself, inasmuch as it appeared to be empty when the pump was used.

OBJECTS OF EXPERIMENTS

The experiments reported in this paper were undertaken to study the effects of (1) fasting and (2) the method of preparation of feed upon the digestive process in dairy cattle, with particular reference to the course of the feed through the stomach.

METHODS OF PROCEDURE

The animals used in these studies were, with one exception, reactors to the tuberculin test. Careful examination of the carcasses by competent veterinarians revealed only very small tubercular lesions; so it is believed that the extent of the tubercular infection was not great enough to cause any abnormal conditions so far as these particular experiments are concerned. The one nonreactor, No. 12313, was a nonbreeder. Details regarding breed, age, and weight are given in Table 1.

Two groups of the animals were fed during preliminary periods of 10 and 14 days, respectively, and the others for as long as conditions permitted. (Table 2.) Three of the cows, Nos. 1, 11, and 742, were milking quite liberally and were therefore fed silage, hay, and a grain mixture, but the others were fed either whole or finely ground alfalfa

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[6] FORBES, E. B., BRAMAN, W. W., KRISS, M., FRIES, J. A., COCHRANE, D. C., JEFFRIES, C. D., SWIFT, R. W., FRENCH, R. B., and MAUCHER, J. V., JR. THE INFLUENCE OF THE ENVIRONMENTAL TEMPERATURE ON THE HEAT PRODUCTION OF CATTLE. Jour. Agr. Research 33: 579–589. 1926.

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[7] FORBES, E. B., KRISS, M., and BRAMAN, W. W. Op. cit. p. 176.

hay as the sole feed. One group of animals was fasted from 4 to 6 days, and then, with the exception of two which were kept as controls, was given feed prepared in various ways shortly before being killed. The other group of animals was not fasted. The time which elapsed between the time of feeding and killing varied considerably for several reasons.

TABLE 1.—*Breed, age, and weight of experimental animals*

Animal No.	Breed [a]	Age at time of slaughter		Live weight just before slaughter	How weight was determined
		Yrs.	*Mos.*	*Pounds*	
1	Crossbred	3	1	900	Estimated by inspection.
11	Grade Holstein	5	6	1,200	Do.
19	do	5	6	1,175	Do.
21	Crossbred	3	5	1,000	Estimated by carcass weight.
26	do	2	0	900	Do.
27	do	2	2	800	Do.
644	Crossbred F_1	4	7	1,000	Estimated by inspection.
718	Crossbred F_2	7	6	1,250	Do.
742	do	4	3	770	Scales.
Guernsey bull	Purebred Guernsey	2	1	1,000	Estimated by carcass weight.
Holstein bull	Purebred Holstein	5	2	2,000	Do.
663	Crossbred F_1	11	7	1,260	Scales.
738	Crossbred F_2	4	7	1,050	Do.
12313	Crossbred	3	9	1,275	Do.

[a] Animals designated "crossbred" were hybrid animals descended from purebred Holsteins and Guernseys.

TABLE 2.—*Character of feed and amount consumed by experimental animals during preliminary period and just previous to slaughter*

FASTED ANIMALS

Animal No.	Preliminary period				Fasting period	Time elapsing between feeding and slaughter	Feed consumed just previous to slaughter	
	Number of days fed	Feed consumed daily					Amount and kind	Dry-matter content [a]
		Silage	Hay	Grain				
		Pounds	*Pounds*	*Pounds*	*Days*	*Hours*		*Kgm.*
1	10	24	[b] 15	8	6	1½	{3.6 pounds ground alfalfa {6.1 pounds ground corn	3.77
11	10	30	[b] 18	8	5	1	10 pounds whole alfalfa	4.00
19	10		[b] 20		5		Not fed	
21	14		[c] 15		4	1½	2,450 gm. dyed ground corn	2.06
26	14		[c] 14		4	1½	10 pounds shelled pop corn	3.82
27	14		[c] 10		4	1½	8.5 pounds ground corn	3.25
644	10		[b] 17		5	1	6 pounds ground alfalfa	2.39
718	10		[b] 20		6	¾	5.5 pounds ground corn	2.10
742	10	(d)	[b](d)	(d)	6	2½	4.4 pounds shelled dent corn	1.68
Guernsey bull.	14		[c] 16		4		Not fed	
Holstein bull.	14		[c] 28		5	14	15 pounds shelled pop corn	5.73

FULL-FED ANIMALS

663	4		[b] 26.5		0	1	About 200 gm. dyed ground corn	0.17
738	4		[b] 23		0	1½	8,454 gm. dyed ground corn	7.10
12313	2		[b] 14.6		0	6	15.1 pounds shelled dent corn	5.76

[a] Dry-matter content of feeds: Ground alfalfa, 87.71 per cent; ground corn, 84.23 per cent; whole alfalfa, 88 per cent (estimated); dyed corn, shelled corn, and pop corn, 84 per cent (estimated).
[b] Whole alfalfa hay.
[c] Finely ground alfalfa hay.
[d] No record of amount kept.

It was planned that all animals, with the exception of the Holstein bull, should be killed as soon as they had finished eating, but on account of the failure of some animals to eat more than a small part of the feed allotted them, the slow eating of others, and various delays encountered in the routine of the slaughtering process, the time elapsing between the commencement of the meal by the animals and the time of killing ranged from about one to six hours. Some animals required about three-fourths of an hour to complete the meal, so that part of the animals were killed within a few minutes after they had finished eating.

The general procedure followed in each instance was to slaughter the animal for meat purposes in the usual manner. After the animal had been partially raised from the floor by means of a hoist attached to the rear legs, the esophagus was severed near the stomach, and in case leakage through this opening appeared likely, it was closed by means of a wire ligature. The stomach and intestines were then removed and taken to one side.

The contents of the rumen were removed first. As the stomach lay upon the floor, a large opening was made in the upper wall of the rumen and the contents scooped into large galvanized-iron tubs by means of a rounded dish. In cases in which the rumen contents were so liquid that the liquid portion separated on standing, this liquid was dished out of the tubs and poured through a fine sieve, thus separating the liquid from the solid portions. Each portion was weighed separately. The solid portion was mixed thoroughly by hand and a sample taken by selecting handfuls at random. The liquid portion was thoroughly stirred and about 1 quart was placed in a half-gallon glass jar provided with rubber ring and glass cover.

The reticulum was next opened and the entire contents saved for analysis.

The omasum was cut away from the other compartments of the stomach, placed on a metal table, sectioned, and the contents removed as completely as possible by hand. The feed residues recovered were weighed and subsampled.

The abomasum was opened and the contents scraped out and saved for analysis.

The intestines were cut into sections and carefully pressed as they were drawn through the hand, thus removing the contents. In most cases—the exceptions being the two bulls and two of the full-fed cows—the entire intestinal contents were collected and dried for analysis.

The methods employed in collection did not yield 100 per cent of the gastrointestinal contents, since small particles adhered to the linings. It is believed that the error involved in this way is relatively small, however. Had water been employed to wash out the various parts of the tract a more nearly perfect collection could have been made, but this would have prevented the determination of the dry matter in the feed residues as they were present, which was considered a point of special interest. Further, the addition of water to the collections would have increased their bulk to such an extent that subsampling would have been necessary in most cases. Accurate subsampling of material containing both free liquid and coarse solid material is difficult. Under the plan followed, only the rumen and omasum contents and seven other samples were sub-

sampled. Another small error which it was not found possible to avoid occurred when the animals fell to the floor after being stunned or shot. In some cases 1 or 2 quarts of liquid containing a small amount of solid matter was regurgitated through the mouth and thrown out over the floor.

The samples of the liquid contents of the rumen were placed in a refrigerator. Dry matter was determined in subsamples removed by means of a pipette. The other samples and subsamples were brought to air-dry condition on the steam bath. They were carefully examined by hand and by means of a magnet, and foreign material such as nails, wire, stones, and cinders removed. With the exception of samples collected from the small intestine, the samples were then ground to pass a 1-mm. sieve and dry matter and crude fiber determined according to the methods prescribed by the Association of Official Agricultural Chemists.[8] It was not found possible to grind the samples of the small intestine contents because they were so sticky. Dry matter was, therefore, determined in these samples by heating the entire sample to dryness in a hot-air oven. These samples were then reduced to the necessary degree of fineness in an iron mortar and crude fiber determined in the usual manner.

EFFECT OF FASTING ON GASTROINTESTINAL CONTENTS

With the exception of Nos. 1, 11, and 742, which were producing milk, the cattle were fed alfalfa hay as the sole feed during the preliminary period. It was planned that this should be consumed at the rate of 1½ pounds daily for each 100 pounds live weight. As shown in Table 2, some of the animals were fed whole hay only, and some finely ground hay.

Considering the relatively low plane of feeding during the preliminary period and the length of the fast, surprisingly large amounts of solid and liquid matter were found in the gastrointestinal tracts of the fasted animals. (Table 3.) The rumen contents of these animals differed from those of the full-fed animals in containing a much larger proportion of liquid. Much of the liquid separated out on standing; with the full-fed animals the rumen contents showed practically no free liquid. Watering the animals shortly before slaughter seemed to have but little effect upon the amounts of liquid in the rumen. Animals Nos. 1, 11, 19, 644, and 718 were offered water on the day of the slaughter; the others were not.

The presence of these large amounts of liquid in the rumens of the fasted animals seemed to indicate that this liquid was either fulfilling the function of distending the rumen to at least part of its normal size and form, or that in the absence of sufficient feed to cause the usual muscular activity of the walls of the rumen the liquid had merely stagnated at that point. The fact that the animals drank less water during fasting than normally, and that they were very quiet and listless after the first day of fasting, gives some support to the latter view. Cow 742 was affected more severely by the fast than the other animals. The last feeding was on the morning of

[8] ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. COMPILED BY THE COMMITTEE ON EDITING METHODS OF ANALYSIS. Revised to July 1, 1924. Ed. 2, 535 p., illus. Washington, D. C. 1925.

March 4, at which time she was also watered. From March 5 to 9, inclusive, she drank only 34 pounds of water. During the last day or two she was unable to rise. She was killed March 10. The other animals seemed normal so far as strength was concerned, although they were much less active while fasting.

TABLE 3.—*Contents of gastrointestinal tracts of dairy cattle*

FRESH BASIS

Animal No.	Contents of—								Total recovered	Total less feed given
	Rumen			Reticulum	Omasum	Abomasum	Small intestine	Large intestine		
	Solid	Liquid	Total							
Fasted animals:	*Pounds*	*Pounds*	*Pounds*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Kgm.*	*Kgm.*
1	87.5	88.5	176.0	[a]	5,561	3,176	4,416	4,320	97.5	93.1
11	94.0	52.0	146.0	1,379	6,909	1,642	3,787	4,535	84.5	80.0
19	36.0	49.0	85.0	458	6,367	1,230	4,279	[b]	57.0	57.0
21	40.0	19.0	59.0	569	4,131	657	2,799	2,271	37.2	34.7
26	50.5	27.5	78.0	[a]	4,347	590	1,861	1,876	44.1	39.6
27	38.0	25.0	63.0	1,272	2,974	[b]	2,235	1,645	37.7	33.8
644	45.0	58.5	103.5	91	4,705	2,085	5,685	3,341	62.9	60.2
718	30.0	9.5	39.5	160	4,947	1,331	2,583	3,066	30.0	27.5
742	32.0	10.5	42.5	111	6,615	2,285	2,095	5,252	35.7	33.7
Guernsey bull	53.5	28.0	81.5	316	6,859	1,466	6,108	7,532	59.3	59.3
Holstein bull	40.0	102.0	142.0	486	5,763	3,964	6,820	5,853	87.4	80.6
Full-fed animals:										
663	103.0	0	103.0	191	10,035	4,334	5,580	9,192	76.1	75.9
738	109.5	0	109.5	2,828	8,395	3,338	5,255	4,134	73.7	65.2
12313	106.0	0	106.0	654	8,078	1,220	3,470	2,519	64.1	57.2

DRY-MATTER BASIS

Fasted animals:										
1	13.4	1.9	15.3	------	907	265	226	340	8.77	5.00
11	12.7	1.3	14.0	232	1,475	236	255	298	8.85	4.85
19	5.4	.6	6.0	113	1,263	156	274	557	5.08	5.08
21	7.2	.4	7.6	135	696	49	160	297	4.78	2.72
26	12.2	.4	12.6	------	925	77	119	264	7.10	3.28
27	7.9	1.0	8.9	236	638	118	164	186	5.38	2.13
644	5.6	1.1	6.7	18	845	261	316	340	4.82	2.43
718	7.3	.3	7.6	47	880	136	148	256	4.91	2.81
742	6.3	.2	6.5	19	1,199	319	136	839	5.46	3.78
Guernsey bull	7.4	.4	7.7	62	1,205	186	410	870	6.28	6.28
Holstein bull	15.9	1.1	17.0	214	987	582	361	412	10.28	4.55
Full-fed animals:										
663	12.6	0	12.6	94	2,175	533	408	1,120	10.05	9.85
738	24.0	0	24.0	700	2,184	408	472	407	15.08	7.97
12313	21.3	0	21.3	303	2,167	236	317	401	13.10	7.32

[a] Combined with rumen contents through error.

[b] Fresh weights lost.

Large amounts of solid matter were also present in the rumens of the fasted animals. Masses of bulky feed weighing 36 pounds and 53.5 pounds, respectively, were recovered from the stomachs of animal No. 19 and the Guernsey bull. These animals had been fasted five and four days, respectively, and were not fed just previous to slaughter, as was the case with the other animals. The total dry matter in the rumens of these animals was 6 pounds and 7.7 pounds, respectively.

Examination of the other parts of the gastrointestinal tracts of the fasted animals revealed the presence of food material resembling in all respects that in the full-fed animals, except that in the fasted animals the amounts, upon the whole, were not so great.

The tracts of the two animals not fed after their fast, No. 19 and the Guernsey bull, contained slightly larger amounts of dry matter

than the computed amounts present in the tracts of the other fasted animals after deduction of the amounts of dry matter in the feed given just previous to slaughter. The total dry matter recovered from the gastrointestinal tracts of the fasted animals, less that in the feed consumed just before slaughter, ranged roughly from one-fourth to two-thirds of that recovered from the full-fed animals. Eighty-five per cent as much dry matter was recovered from the tract of the Guernsey bull, fasted four days and weighing about 1,000 pounds, as from No. 12313, full-fed and weighing 1,275 pounds.

TABLE 4.—*Percentage of dry matter in contents of gastrointestinal tracts of dairy cattle*

FASTED ANIMALS

Animal No.	Percentage of dry matter in contents of—					
	Rumen	Reticulum	Omasum	Abomasum	Small intestine	Large intestine
1	8.69		17.93	8.35	5.12	7.88
11	9.59	16.84	21.35	14.36	6.73	6.57
19	7.06	24.57	19.83	12.72	6.40	
21	12.88	23.68	16.85	7.48	5.72	13.08
26	16.15		21.29	13.07	6.39	14.07
27	14.13	18.55	21.45		7.32	11.33
644	6.47	19.73	17.96	12.51	5.56	10.18
718	19.24	29.07	17.79	10.22	5.73	8.35
742	15.29	17.44	18.12	13.95	6.49	15.98
Guernsey bull	9.57	19.72	17.57	12.66	6.72	11.55
Holstein bull	11.97	44.03	17.13	14.67	5.29	7.04

FULL-FED ANIMALS

663	12.23	49.23	21.67	12.30	7.32	12.18
738	21.92	24.74	26.01	12.21	8.99	9.84
12313	20.09	46.37	26.83	19.36	9.14	15.93

TABLE 5.—*Percentage of total contents of gastrointestinal tracts found in each division (dry-matter basis)*

FASTED ANIMALS

Animal No.	Percentage of total gastrointestinal content in—					
	Rumen	Reticulum	Omasum	Abomasum	Small intestine	Large intestine
1	79.2		11.4	3.0	2.6	3.9
11	71.9	2.6	16.7	2.7	2.9	3.4
19	53.7	2.2	24.9	3.1	5.4	11.0
21	72.2	2.8	14.6	1.0	3.3	6.2
26	80.6		13.0	1.1	1.7	3.7
27	75.1	4.4	11.9	2.2	3.0	3.5
644	63.1	.4	17.5	5.4	6.6	7.1
718	70.3	1.0	17.9	2.8	3.0	5.2
742	54.0	.3	22.0	5.8	2.5	15.4
Guernsey bull	56.4	1.0	19.2	3.0	6.5	14.0
Holstein bull	75.2	2.1	9.6	5.7	3.5	4.0

FULL-FED ANIMALS

663	56.9	0.9	21.6	5.3	4.1	11.1
738	72.3	4.6	14.5	2.7	3.1	2.7
12313	73.9	2.3	16.5	1.8	2.4	3.1

A notable feature of the recoveries was that the digestive processes seemed to be proceeding in a normal manner in the omasums, abomasums, and small intestines of the fasted animals, as judged by the percentage of dry matter in the contents of these organs (Table 4), and by the proportion of the total content of the gastrointestinal tracts found in each division (Table 5). While there was considerable variation in the percentages of dry matter in the contents of the different divisions, the results are consistent in showing a close resemblance between the fasted and full-fed animals. It is interesting to note in this connection the relatively high dry-matter percentage in the reticulum contents, indicating that, as recovered in the usual methods of slaughter, this compartment of the stomach is not a "water bag." Further, with but one exception, the omasum contents had higher dry-matter percentages than the contents of the other divisions of the tract except the reticulum. The percentage of dry matter in the reticulum contents would fall below that of the omasum contents in several other instances if computed after deduction of the foreign matter (nails, wire, etc.). The small intestine contents were quite liquid in consistency, the percentage of dry matter in all cases being less than 10 per cent. It is possible that the rather high dry-matter percentage in the large intestine contents of the fasted animals was, in part, due to stagnation of the feed residues. It was noted at the time of recovery that this apparently had occurred in cow 742, an animal which was inactive during her last few days.

Further support for the theory that digestion was proceeding in a fairly normal manner in the fasted animals is shown in Table 5. The proportion of the contents of the total gastrointestinal tracts found in the different divisions of the control animals, No. 19 and the Guernsey bull, were not greatly different from those of the other animals, both fasted and full-fed, and indicated that each division contained about its normal share of feed or feed residues.

The feces of three of the animals were collected during the fasting period, the collections beginning about 10 hours after commencement of the fast. The data in Table 6 show that there was some irregularity in excretion, possibly due in part to the close confinement of the animals in stalls, but that upon the whole, digestion, as indicated by elimination of feed residues, was proceeding at an active rate up to and including the fourth day of fasting.

TABLE 6.—*Dry matter voided in feces during fasting*

Animal No.	Date	Length of collection period	Fresh weight of feces	Dry matter	Dry matter voided daily
		Hours	*Grams*	*Per cent*	*Grams*
21	Nov. 9, 1926	14½	4,921	25.8	1,280
21	Nov. 10, 1926	24	4,108	22.0	904
21	Nov. 11, 1926	24	2,228	26.2	584
21	Nov. 12, 1926	24	2,169	27.4	594
26	Nov. 9, 1926	14½	2,405	21.4	515
26	Nov. 10, 1926	24	2,539	21.6	548
26	Nov. 11, 1926	24	2,465	20.8	513
26	Nov. 12, 1926	24	1,683	22.0	370
27	Nov. 9, 1926	14½	2,560	24.8	635
27	Nov. 10, 1926	24	4,344	17.2	747
27	Nov. 11, 1926	24	2,635	14.6	385
27	Nov. 12, 1926	24	2,404	13.8	332

EFFECT OF METHOD OF PREPARATION OF FEED ON GASTRO-INTESTINAL CONTENTS

The alfalfa hay fed during the preliminary period and just previous to slaughter, was fourth-cutting western hay, containing about 55 per cent leaves and having a high green color. The color was so pronounced that the gastric contents of animals fed hay only were of a bright green color. This made it very easy to determine the location of the corn fed just previous to slaughter.

It was not possible to make quantitative separations of the feed consumed just before slaughter and that already in the stomach, chiefly on account of the intimate mixing of the rumen contents which it was found had taken place. Therefore, in all but one or two cases in which separations were attempted, observations were made and notes taken regarding the approximate proportions of the recently consumed feed found in the different divisions.

The animals fed whole alfalfa hay during the preliminary period retained amounts of dry matter in their rumens ranging from about 1.5 to 7 pounds. This calculation is based upon the assumption, made merely for purposes of comparison, that all of the feed consumed just before slaughter remained in the paunch. These amounts are no greater than those in the case of the animals fed the finely ground hay.

If it be assumed, as Armsby [9] implies, that "finely comminuted portions probably pass on directly to the omasum, or manifolds, and the abomasum," then cattle fed on finely ground hay for 14 days and subsequently fasted for 4 or 5 days—as was the case with animals 21, 26, 27, and the Guernsey and Holstein bulls—should have no alfalfa residues in the rumen. In these experiments, however, all of these cattle had considerable amounts of hay residues in their rumens. In the case of the Guernsey bull, the hay residues contained 7.7 pounds of dry matter, while in the other cases the dry-matter content ranged from about 1 to 4 pounds.

There seemed to be little difference between the animals fed whole hay and those fed ground hay, with respect to the amounts of dry matter of the rumen contents after deducting the amounts in the feed eaten just before slaughter. Furthermore, no differences in the amounts of feed which might be attributed to the method of preparation of the hay could be observed in the other parts of the alimentary canal. The ground alfalfa consumed by Nos. 1 and 644 was recovered chiefly in their rumens.

Referring again to Armsby's statement regarding the course of the finely comminuted feed, it would be inferred that if his assumption is correct, finely ground corn would not enter the paunch, or at least most of it would not. Amadon's [10] theory that the course of the feed is determined largely by its weight would seem to imply that a portion of a heavy feed, such as ground corn, would enter the reticulum directly.

Most of the corn fed was recovered in the rumens and reticula, except in the case of the Holstein bull. This was true whether the corn was fed as shelled corn or ground corn. Shelled pop corn was fed to two animals upon the assumption that few of these kernels

[9] ARMSBY, H. P. Op. cit.

[10] AMADON, R. S. Op. cit.

would be broken by the animals in eating, which proved to be the case. Some coarsely ground yellow corn was dyed a brilliant red color by treating it with Congo Red 4B in water at a temperature of 110°–120° F., using about 1 gm. of the dye per pound of corn. After standing about four hours, the corn was washed by decantation until the wash water was nearly clear, dried in shallow pans on the steam bath, and reground. The dye did not color perceptibly the other alimentary contents of the animals consuming the dyed corn.

It was very easy to distinguish the bright red corn mixed with the green hay residues. One of the most surprising features in connection with the recovery of the rumen contents was the thorough mixing of the newly eaten feed with the residues already in the rumen, which had occurred during the relatively short intervals between feeding and killing. The mixing had been done almost as thoroughly as could be done by hand or by means of a mechanical mixer. A slight exception to this general condition was found in the case of No. 738, fed a large amount of ground corn. Here the corn was intimately mixed with the hay residues, as in the other animals, except that 100–200 gm. of corn was found practically unmixed in the lower part of the rumen sacs. This was but a small part of the whole, however.

In the case of animals fed large amounts of ground corn, notably Nos. 27 and 738, a considerable quantity was recovered in the reticula, although this was not over one-third of that fed. A thorough mixing of the contents of this compartment had occurred also, and the corn formed a larger proportion of the feed present than in the rumen.

In but few cases was any of the feed consumed just before slaughter found in the omasum. In cow 718 some ground corn was present in the reticulum, but none in the omasum or abomasum. Animal No. 27 had a rather large quantity of corn in the reticulum but only about 1 ounce in the omasum. In No. 21 a very small amount of dyed corn was noted along the lower edges of the leaves of the omasum. In the case of cow 738, the bright red corn was mixed with the hay along the ventral side of the omasum and had spread out from the opening about one-fifth to one-fourth of the distance from the opening to the opposite side of the compartment. No corn was found in the abomasum. Cow 663 refused to eat more than part of a pound of her feed, and it was very difficult to trace this small amount accurately after it had become mixed with the large mass of hay residues present. None of the corn was noted in the omasum, however.

Ten pounds of shelled pop corn was fed to No. 26 about one and one-half hours before slaughter. No broken kernels were observed. The bulk of the corn was found in the rumen, with a considerable quantity in the reticulum. Only a few kernels (10 to 15) were noted in the omasum and none were recovered in the abomasum.

Heifer 12313 was fed 15.1 pounds of shelled corn six hours before she was killed. Most of this was recovered in the rumen. After air drying the samples of material recovered from the alimentary tract, the corn kernels were separated and weighed. Calculating the recovery in the samples upon the basis of the total contents of the different divisions, the corn recovered formed 79 per cent of that fed, 70 per cent being found in the rumen, 3 per cent in the reticulum, 4 per cent in the omasum, and 2 per cent in the abomasum. About

4 gm., or 0.1 per cent, were also found in the large intestine, but it is believed that this, as well as part of that found in the abomasum, was corn from silage fed previous to the two-day preliminary period.

The Holstein bull was fed 15 pounds of shelled pop corn the evening before slaughter. The chief solid contents of the rumen and reticulum of this animal consisted of pop corn. The small amount of other solid material present consisted chiefly of bedding material and some hay. No bedding material was recovered in the other animals. Hay residues were present in the omasum, however. As in the case of the other animals fed shelled corn, most of the corn fed this animal was recovered in the rumen, with a portion in the reticulum. In the omasum the kernels of corn were quite generally distributed through the leaves along the ventral side, but had not yet reached the dorsal portion. A few ounces of corn were recovered in the abomasum. The corn had progressed farther into the omasum than in the case of the other animals.

TABLE 7.—*Fineness of grinding of feeds given during preliminary period and just previous to slaughter*

Diameter of sieve openings	Proportion of sample retained on sieves		Diameter of sieve openings	Proportion of sample retained on sieves	
	Alfalfa hay	Shelled corn		Alfalfa hay	Shelled corn
Mm.	*Per cent*	*Per cent*	*Mm.*	*Per cent*	*Per cent*
5	0	0	1	60	28
3	0	0	0.5	29	27
2	4	3	Bottom pan	7	42

The mixture of ground corn and ground hay fed to animal No. 1 was recovered chiefly in the rumen. Both these feeds were very finely ground, as shown in Table 7. All of the hay used in the experiment, including that in the preliminary period, whether fed in whole or ground form, was originally of the same lot and quite uniform in quality. The analyses of these feeds are shown in Table 8.

The muscular movements of the rumen seemed to have been much more active in the full-fed than in the fasted animals, as judged by the completeness of mixing of the feed recently eaten with that already in the rumen.

TABLE 8.—*Composition of feeds used*

Kind of feed	Dry matter	Ash	Crude protein	Crude fiber	Ether extract	Nitrogen free extract
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Ground corn	84.23	1.50	9.21	2.11	2.22	69.19
Ground alfalfa	87.71	9.58	16.64	26.37	2.30	32.82

TABLE 9.—*Crude-fiber content of material removed from gastrointestinal tracts*

FRESH BASIS

Animal No.	Crude fiber in material removed from—					
	Rumen [a]	Reticulum	Omasum	Abomasum	Small intestine	Large intestine
Fasted animals:	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1	4.61	----	9.64	3.92	0.54	4.12
11	4.98	8.16	8.07	4.64	.15	2.76
19	4.29	10.32	9.79	4.78	1.17	----
21	3.12	4.16	7.50	2.82	1.24	5.34
26	3.05	----	9.24	2.59	.63	5.61
27	2.53	2.94	6.75	----	1.29	4.23
644	3.67	9.43	9.87	7.19	1.54	5.63
718	5.72	6.40	9.13	5.02	.89	4.03
742	4.68	5.60	6.84	3.09	.42	4.80
Guernsey bull	5.52	9.95	7.34	3.97	1.52	4.51
Holstein bull	1.00	4.73	7.25	3.99	.61	2.84
Full-fed animals:						
663	5.90	7.94	8.31	4.24	.90	4.73
738	4.54	5.41	8.84	4.66	1.55	3.55
12313	5.41	4.00	8.62	3.77	1.04	3.97

DRY-MATTER BASIS

Fasted animals:						
1	53.08	----	53.76	46.91	10.63	52.28
11	51.92	48.46	37.82	32.33	2.17	41.99
19	60.76	42.01	49.35	37.56	18.35	46.53
21	24.24	17.57	44.53	37.65	21.74	40.81
26	18.91	----	43.39	19.85	9.92	39.89
27	17.91	15.83	31.47	29.89	17.69	37.35
644	56.76	47.81	54.95	57.44	27.78	55.31
718	29.75	22.00	51.33	49.10	15.52	48.32
742	30.63	32.12	37.73	22.12	6.46	30.04
Guernsey bull	57.70	50.47	41.78	31.37	22.64	39.01
Holstein bull	8.37	10.75	42.33	27.20	11.58	40.27
Full-fed animals:						
663	48.26	16.13	38.37	34.47	12.23	38.81
738	20.72	21.87	33.99	38.15	17.22	36.06
12313	26.95	8.63	32.11	19.45	11.43	24.91

[a] The calculations in this column are based upon crude-fiber determinations of the samples of the solid portion of the rumen contents. The relatively small amounts of dry matter in the liquid portion of the rumen contents are not included.

CRUDE FIBER IN GASTROINTESTINAL CONTENTS

The percentages of crude fiber in the gastrointestinal contents (Table 9), when calculated upon the fresh basis, furnish little or no information regarding the nature of the material, owing chiefly to differences in the percentages of dry matter. The percentages of crude fiber in the omasum contents of the different animals are upon the whole higher than those in the contents of the other divisions and show some degree of similarity, the range being roughly from 7 to 10 per cent. The crude fiber percentages in the small intestine contents are low, all being less than 2 per cent.

When calculated in terms of the percentages of the dry matter comprised by crude fiber, some interesting figures were obtained. The two highest crude-fiber contents in the rumen samples were those of cow 19 and the Guernsey bull, the animals not fed after the fasting period. These high percentages are about twice as great as the crude-fiber content of the hay fed during the preliminary period. (Table 8.) Since the feed residues recovered from these animals appeared to consist of alfalfa hay only, it is assumed that the more readily soluble

portions of the hay had been removed in the processes of digestion, so that the residues remaining in the rumen consisted largely of crude fiber. Further support for this theory is found in the data for Nos. 11 and 644, fed alfalfa hay only, which show high percentages of fiber in the rumen contents. Then, too, these crude-fiber percentages correspond roughly to those of the large intestine contents, from which presumably most of the digestible material had been absorbed.

All the animals fed corn shortly before slaughter, with the exception of Nos. 1 and 663, show distinctly lower percentages of fiber in the rumen contents (dry-matter basis) than the other animals. The presence of the corn was undoubtedly responsible for these striking differences. Cow 663 ate only 200 gm. of corn, which could have had but iittle effect in lowering the fiber in the rumen contents. The quantity of fiber in her case corresponds to that of the animals fed hay only. No explanation is at hand for the high percentage of fiber in the rumen of animal No. 1, fed both hay and corn.

The quantity of fiber present in the reticula of these animals corresponds in a general way to that in the rumens. The animals not fed or fed hay only have high proportions of fiber in both rumens and reticula, whereas those fed corn in considerable quantity have low percentages of fiber in the contents of both compartments.

The crude fiber in the omasum contents is high, although there is a rather wide variability. Corn was found in but few of the omasums and then in but small amounts, the contents apparently consisting chiefly of alfalfa residues. Possibly the differences in the fiber contents represent differences in the completeness of absorption of the more soluble constituents of the hay.

Considerable variability is shown in the crude-fiber percentages of the abomasum contents, although in general these are high.

Crude fiber formed a suprisingly low proportion of the contents of the small intestine. Possibly this may be accounted for by the presence of mucus, salts, and digestive juices.

The contents of the large intestine contained a large proportion of crude fiber, except in the case of No. 12313. The small percentage in this instance may have been caused by the mixed ration fed previous to the two-day preliminary period. Some kernels of corn were noted in the contents of the abomasum and large intestine.

Considerable difficulty was encountered in obtaining checks of duplicate determinations of crude fiber owing to the presence of sand and like material in the samples. The female animals had access to steamed bone meal and finely ground limestone, provided in self-feeders, and the presence of pebbles and cinders in the abomasums indicated that they may have consumed some dirt about the yards.

Of the 82 determinations of crude fiber in the air-dry material, 35 of the determinations made in duplicate checked within 0.4 per cent, 23 differed from 0.45 to 0.80 per cent, and the remainder differed from 0.85 to 1.3 per cent.

FOREIGN MATTER IN GASTROINTESTINAL CONTENTS

Foreign matter, such as nails, wire, glass, and stones, was found in the stomachs of all the experimental animals. The amounts recovered from each division are shown in Table 10. With the exception

of the two cases in which the reticulum contents were included with the rumen contents through error, foreign substances were present in all reticula and abomasums and in a few cases in the rumens. No foreign bodies of perceptible size were found in the omasums, small intestines, or large intestines, although considerable sand or similar material was present in some of these. The foreign matter found in the reticula consisted chiefly of nails, wire, glass, and sand (fig. 1), while in the abomasums it was largely small pebbles and cinders (figs. 2, 3, and 4). These findings indicate a movement of at least a part of the foreign material through the stomach. The statement of Amadon [11] that "whole corn kernels are passed on through the digestive system, but there is no evidence to show that such is the case as regards the foreign bodies, nails, etc.," is not therefore wholly in agreement with the results here shown.

FIG. 1.—Foreign matter removed from the reticulum of animal No. 11. The nature of this material is typical of that recovered from the reticula of the animals in these experiments

TABLE 10.—*Amounts of foreign matter removed from gastrointestinal tracts of dairy cattle*

Animal No.	Foreign matter removed from—					
	Rumen [a]	Reticulum	Omasum	Abomasum	Small intestine	Large intestine
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
1	[b] 7		0	16	0	0
11	0	25	0	51	0	0
19	5	40	0	40	0	0
21	0	10	0	2	0	0
26	0		0	2	0	0
27	1	6	0	15	0	0
644	0	9	0	3	0	0
718	7	2	0	3	0	0
742	0	2	0	13	0	0
Guernsey bull	0	19	0	7	0	0
Holstein bull	3	30	0	52	0	0
663	0	60	0	24	0	0
738	0	10	0	31	0	0
12313	0	27	0	15	0	0

[a] Figures show the amount of foreign matter recovered from subsamples. The dry matter of the subsamples formed but a small proportion of the total dry-matter content of the rumen; in the case of No. 1, the percentage being 5.6; for No. 19, 12.1 per cent; No. 27, 13.7 per cent; No. 718, 11.9 per cent; and for the Holstein bull, 10.5 per cent.

[b] May have come from reticulum, since reticulum contents were combined with those of rumen through error.

[11] AMADON, R. S. Op. cit.

THE RUMEN AS A STOREHOUSE

Statistical constants were derived from the data of Table 5, with the object of studying the variability of the data showing the proportion of the total dry matter of the gastrointestinal contents found in each division of the tract. It is shown in Table 11 that these data, upon the whole, have a high degree of variability. This is to be expected on account of the differences in the amounts and character of the feed ingested. Even with these wide differences in feed intake,

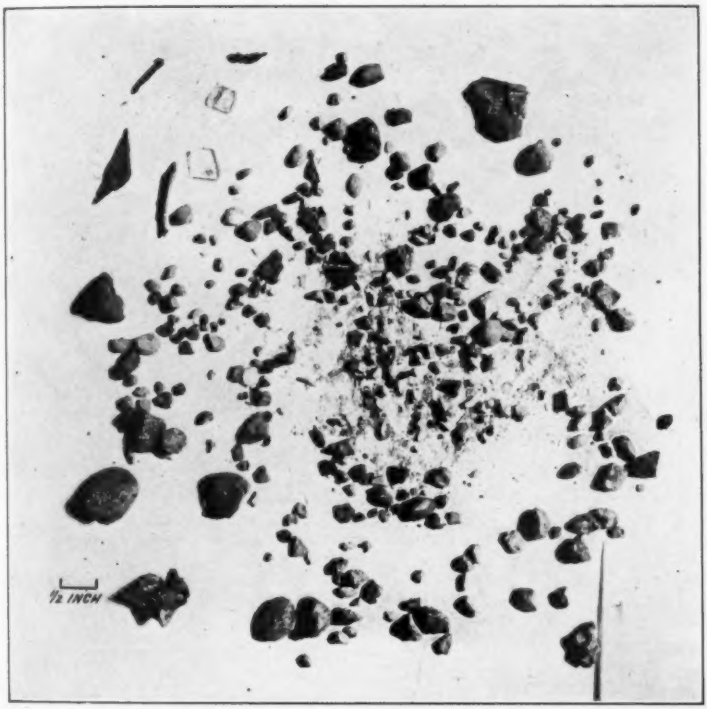

FIG. 2.—Foreign matter recovered from the abomasum of animal No. 11. This matter, which consisted chiefly of stones and cinders, was typical of that secured from the abomasums of all the animals. In this particular instance pieces of glass and metal were present also

however, it is remarkable that the variability of the fasted group so closely corresponds to the variability of all animals taken as a group.

The data for the rumen contents show a smaller variability than those for any of the other divisions. The statistical constants for this compartment indicate that the rumen retains a rather constant proportion of the total dry matter of the gastrointestinal contents. One function of the rumen, therefore, seems to be to act as a storehouse, helping to regulate the rate of passage of food to the other compartments of the stomach.

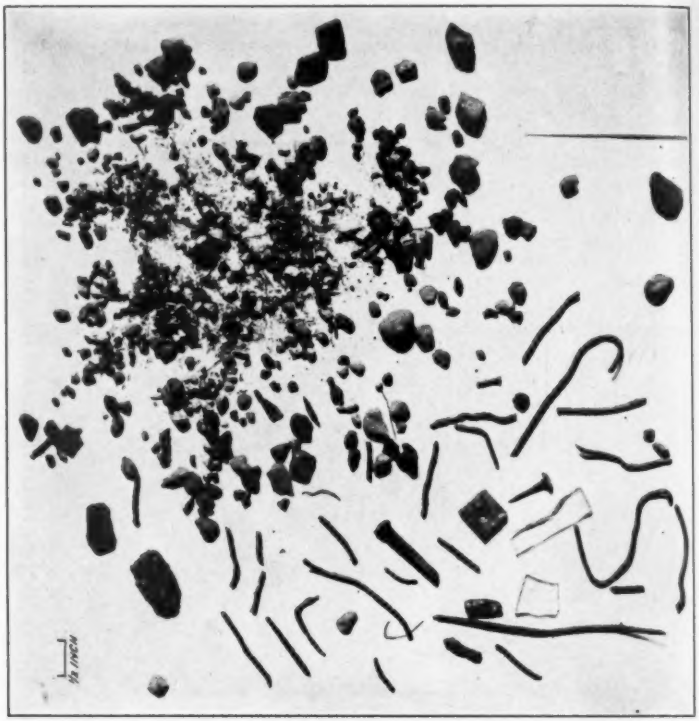

FIG. 3.—Foreign bodies separated from the gastric contents of cow No. 19. Besides numerous sma stones and cinders, pieces of wire and glass, a tack, a burr, and a bullet were found

TABLE 11.—*Statistical constants of the proportion of total contents of gastrointestinal tracts found in each division (from Table 5)*

FASTED ANIMALS

Division of tract	Statistical constants		
	Mean	Standard deviation	Coefficient of variability
Rumen	68. 54±0. 019	9. 54±0. 014	13. 92±0. 394
Reticulum	1. 80± . 003	1. 24± . 002	68. 89±1. 072
Omasum	16. 05± . 010	4. 68± . 001	29. 16± . 173
Abomasum	3. 20± . 0003	1. 62± . 0002	50. 63± . 5227
Small intestine	3. 66± . 0003	1. 59± . 0002	43. 44± . 381
Large intestine	7. 02± . 009	4. 26± . 006	60. 68± . 747

ALL ANIMALS

Rumen	68. 28±1. 671	9. 26±1. 181	13. 56±0. 332
Reticulum	2. 01± . 263	1. 35± . 186	67. 16± . 579
Omasum	16. 32± . 783	4. 34± . 554	26. 59±1. 275
Abomasum	3. 22± . 286	1. 59± . 202	49. 38± . 440
Small intestine	3. 54± . 265	1. 45± . 187	40. 96±3. 110
Large intestine	6. 70± . 767	4. 26± . 542	63. 58±7. 295

SUMMARY AND CONCLUSIONS

Dairy cattle 2 years old or over were fasted from four to six days. The gastrointestinal tracts of these animals contained from one-fourth to two-thirds as much dry matter as those of animals full-fed up to the time of slaughter. Large amounts of free liquid were present in the rumens of the fasted animals, whereas little or none was present in the rumens of the unfasted cattle. Digestion seemed to have been proceeding in a normal manner (except for quantity) in the fasted animals, as judged by the percentages of dry matter

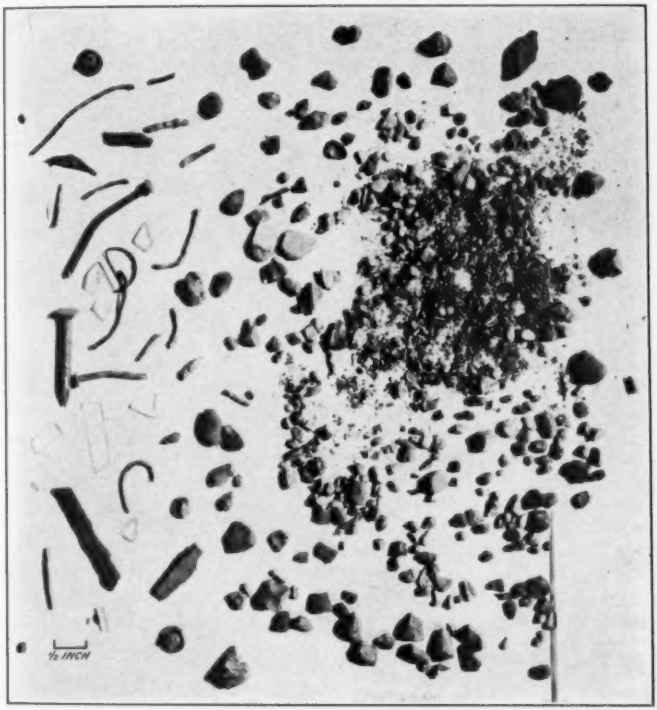

FIG. 4.—Materials found in the stomach of the Holstein bull. In this and other cases, some of the pieces of metal appeared to have been flattened, as if by the buhrs of a feed grinder. The heavy nail shown in the lower part of the illustration was found in the rumen

and the proportions of the total contents found in each division of the gastrointestinal tract and by the output of dry matter in the feces.

Three animals were fed only whole alfalfa hay during a 10-day preliminary period previous to fasting, while five animals were fed only finely ground alfalfa hay for 14 days previous to fasting. The form in which the hay was fed seemed to have no effect upon the amount of dry matter in the rumens.

The larger part of both finely ground and shelled corn fed to different animals shortly before slaughter was recovered in the rumens,

where it had been thoroughly mixed with the other material present. A small portion of the corn was found in the reticula, while in a few cases some corn was found in the omasums. The method of preparation of the corn seemed to bear no relation to the path it followed through the stomach. The mixing of ground corn and ground hay before feeding (one animal only) seemed to yield no different results than when these feeds were given separately.

Crude fiber formed a high percentage of the dry matter in the stomach contents of the animals fed alfalfa only, while it formed a distinctly lower proportion in the rumen and reticulum contents of most of the animals which were fed corn shortly before being killed. On account of the high percentages of crude fiber in the rumen contents of the animals fed alfalfa only and then fasted, which correspond roughly to the crude-fiber contents of the reticulum, omasum, and large intestine, the theory is advanced that in these cases removal of the more digestible and soluble portions of the alfalfa took place in the rumen. Insufficient data are at hand to show whether a similar action occurred in the full-fed animals.

Foreign matter was found in the reticula and abomasums of all animals in which the contents of these divisions were recovered, indicating a movement of a part of such material at least through the digestive tract.

A function of the rumen seems to be that of a storehouse which, as judged by the coefficient of variability, retains a fairly constant proportion of the dry matter of the total gastrointestinal contents, even with a highly variable feed intake.

THE RELATION OF ENVIRONMENT TO SHAPE OF FRUIT IN CUCUMIS SATIVUS L. AND ITS BEARING ON THE GENETIC POTENTIALITIES OF THE PLANTS [1]

By VICTOR A. TIEDJENS

Assistant Research Professor of Vegetable Gardening, Massachusetts Agricultural Experiment Station [2]

INTRODUCTION

Genetic data on size and shape of fruit in *Cucumis sativus* L. are subject to much criticism unless corrected for the influence of environment and plant vigor on the development of the fruit. The difficulty of securing reliable data on shape and size of fruit in cucumber-inheritance studies suggested a number of experiments to determine what effect time of pollination, amount of pollen, soil nutrients, light, and maturing fruits would have on the final shape of newly pollinated flowers.

Commercial growers are very much concerned over the production of large proportions of "seconds" and "nubbins" under various conditions of environment. It is a common phenomenon that a variety produces different shaped fruits under different conditions of environment, and often a variety is condemned for producing poorly shaped fruits when in reality the shape was determined by conditions which have nothing to do with the genetic possibilities of the variety.

Many types of fruit shapes have been isolated through self-fertilization. The establishment of apparently homozygous strains for shape of fruit shows that certain types may be represented as definitely genetic entities. Such shapes which have been definitely fixed may be characterized as wasp, long necks, square shoulders, pointed tips, and those more or less wedge-shaped or cylindrical.

In Figure 1, A, the fruits in the top row represent genetic "wasps," having no ovules in the embryo sacs of the basal portion of the fruit. The bottom row represents physiological wasps in which all the separate seed cavities contain either seeds or rudimentary embryos.

The hereditary types do not concern us in this paper. There are, however, a number of deviations in shape from a normal variety, genetically fixed for shape of fruit, which are discussed. Many irregular types are found on vigorous vines which genetically should produce ideal fruit.

IMPORTANCE OF LOCATION OF PISTILLATE FLOWERS

The first set of fruit on healthy, vigorous vines is heavier than the plant can successfully mature, and only the fruits [3] which mature earliest represent ideal types for the variety. The late-maturing fruits may deviate in various degrees from the normal, depending on the time of pollination, interruptions in the growth cycle, and

[1] Received for publication Mar. 7, 1928; issued July, 1928. Published with permission of the director of the Massachusetts Agricultural Experiment Station.

[2] Thanks are due Prof. W. D. Whitcomb for his helpful criticisms in the preparation of this manuscript.

[3] The term "fruit" is used to designate the stage between fertilization and maturity.

vigor of the plant. Pistillate flowers are produced on a plant until the fruit from the first pollinated flower attains sufficient size to exert an inhibitory effect, when many flowers which have not reached anthesis dry up. If growth is inhibited after a flower is pollinated and fertilization has taken place, the ovary [4] may remain on the

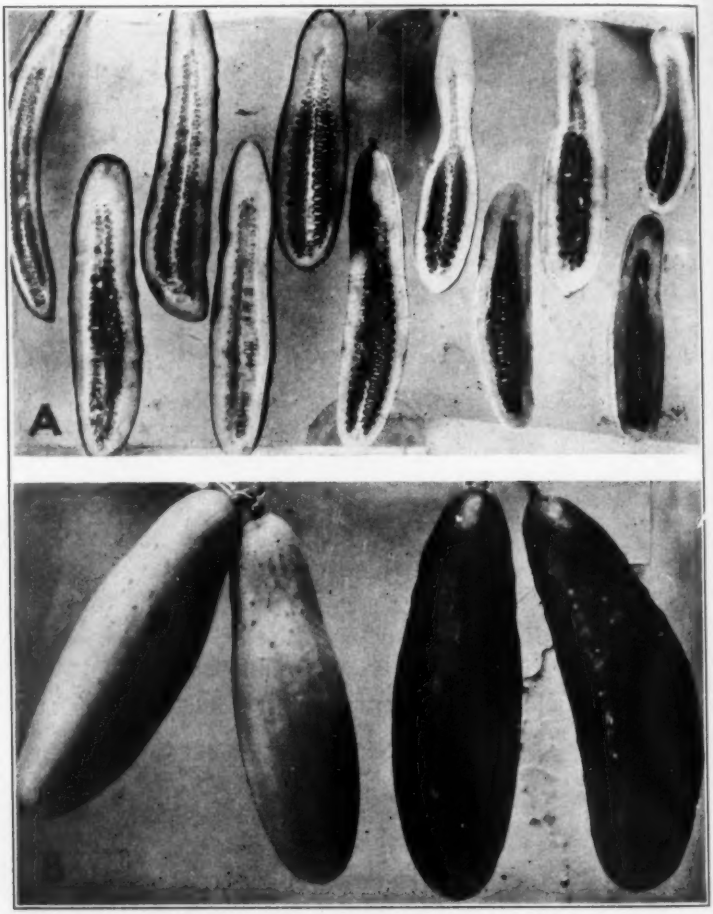

FIG. 1.—A, Cucumber fruits showing genetic "wasps" (top row) which have no ovules in the embryo sacs of the basal portion; physiological "wasps" (bottom row) the seed cavities of which contain seeds or rudimentary embryos; B, two pairs of morphological twins which developed as a result of the simultaneous pollination of two flowers produced at a single node

vine for a long period without increasing in size, but when conditions become favorable it will begin to grow. The effect of such interruptions depends on the type of plant. Some plants produce pistil-

[4] "Ovary" is used to designate the part of the flower that forms the cucumber from the time it is distinguishable in the bud until fertilization takes place, which is usually five or six days after anthesis.

late flowers on practically every node while others produce them only on the nodes of the laterals, and then rather sparingly. The latter type produces a greater proportion of well-shaped fruit because the set is much lighter, although the total yield may be practically the same.

If it happens that two flowers are produced at a node at one time, and are pollinated simultaneously, they develop morphological twins. Two pairs of such twins are shown in Figure 1, B. These are from

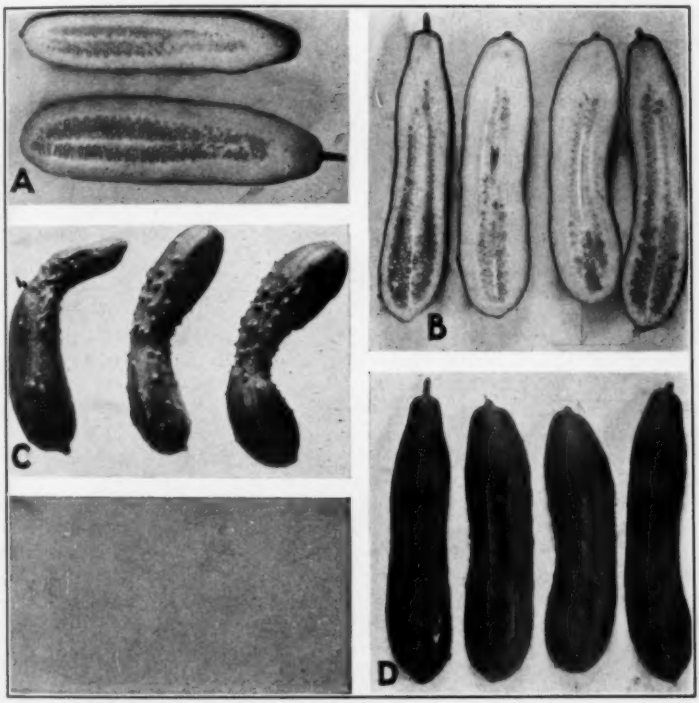

FIG. 2.—Cucumber fruits which developed from flowers pollinated 24 hours after anthesis. All are wasp-shaped. Those shown in A may be classed as first grade but not fancy; those shown in B, C, and D are seconds or culls

two varieties having well-shaped fruit of medium length. The uniformity of the twins is characteristic but their shape and size do not conform to the variety type.

EXPERIMENTAL DATA

EFFECT OF TIME OF POLLINATION ON SHAPE OF FRUIT

To obtain some definite information on the interrelation of fertility of the soil, time of pollination, and shape of fruit, two beds of 50 plants each were planted to a known variety of greenhouse cucumber. The plants were all permitted to mature a few fruits of the first set so that the available nitrogen in the soil would be materially reduced. As soon as the plants showed signs of decreasing vigor, apparently

due to a lack of nitrogen, bed No. 1 was given a copious supply of nitrate of soda; bed No. 2 received no additional nutrients. On April 16, when the nitrate of soda was applied, all the maturing fruits and fertilized ovaries were removed from the vines. All unopened flowers were covered with glassine paper bags and hand-pollinated. On 25 plants in each bed the flowers were pollinated at anthesis, while on the remaining plants the flowers were pollinated 24 hours after anthesis. At the end of 14 days the first pollinated flowers had formed fruits ready to pick for market purposes. The results may be summarized as follows:

Bed No. 1. Nitrate of soda added.

A. Flowers pollinated at anthesis. The first 100 fruits were normal.

B. Flowers pollinated 24 hours after anthesis. The fruits were all wasps, although sufficient tissue had been produced in the constricted portion so that they could be classed as first grade but not fancy. The types shown in Figure 2, A, are characteristic. Seeds developed in one-half to two-thirds of the embryo sacs.

Bed No. 2. No nitrate of soda added.

A. Flowers pollinated at anthesis. Sixty-three out of the first 100 fruits were practically normal while the others showed sufficient irregularities to be classed as seconds or culls. Seven of the 100 were bad wasp types, and very much under size.

B. Flowers pollinated 24 hours after anthesis. All of the fruits were wasp shaped, varying from very short to normal length. All were seconds or culls. The fruits shown in Figure 2, B, C, and D, were common types.

Late pollination apparently accomplishes fertilization in the ovules of the tip of the ovary but not in the ovules toward the base. Sufficient tissue is produced to inclose the seed cavity, and only when the plant receives ample nutrients to fill out the fruit does it resemble the ideal for the variety.

As a result of the above observations further studies on pollination were conducted. A few preliminary notes are of interest.

The flowers on a cucumber plant are fully open between 2 and 4 o'clock in the morning at a temperature of 16° C. At 12° the flowers open very slowly and reach their maximum between 8 and 9 o'clock in the morning. Thus the time of anthesis can be controlled by the temperature.

Germination studies showed that pollen loses its vitality by 2 o'clock in the afternoon at temperatures of 27° C. in bright sunlight. Lower temperatures extend the time somewhat.

An attempt was made to bring about fertilization by pollinating at various times before and after anthesis. The night temperature was kept at or above 14° C. during the experiment. All pollinations, designated "at anthesis," were made between 6 and 8 o'clock in the morning, which was a few hours after anthesis for some flowers. The soil was sufficiently fertile to produce well-shaped fruits with ordinary pollination. The fruits and fertilized ovaries were all removed previous to starting the experiment and all unopened flowers were bagged. Although the results as shown are more or less fragmentary, they are indicative of the effect of time of pollination on the shape of the fruit.

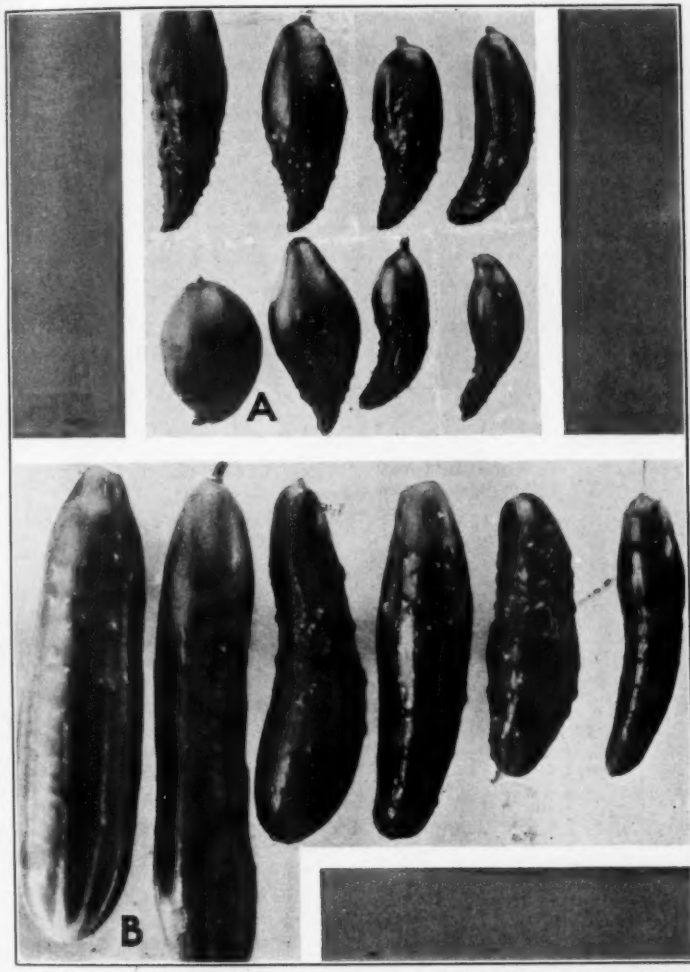

FIG. 3.—A, Nubbins produced by cucumber vines in poor vigor; B, stunted fruits, showing the detrimental effect caused by a growing fruit on the development of later-pollinated flowers

Pollen taken from staminate flowers—	Pollen deposited on pistillate flowers—	Percentage of pollinations successful	Shape of fruit
5 hours after anthesis	28 hours after anthesis	77	All wasp shapes.
Do	5 hours after anthesis	100	All first grade or fancy.
Do	18 hours before anthesis	70	All first grade.
Do	42 hours before anthesis	7	Do.
20 hours before anthesis	5 hours after anthesis	83	Do.
12 hours before anthesis	24 hours after anthesis	67	All wasp shapes.
20 hours before anthesis	20 hours before anthesis	0	
14 hours after anthesis	14 hours after anthesis	0	
8 hours after anthesis	8 hours after anthesis	92	17 wasp shapes out of 50 fruits.

The results show the possibility of using flowers before anthesis where a limited number of plants are being employed for genetic studies. Even though pollination is made some time before anthesis, the germination of the pollen probably does not take place until shortly before the beginning of anthesis when the sugary secretion is produced. The age of the pollen then determines whether fertilization will take place and may account for the low percentage of set where the pistillate flower was pollinated 42 hours before anthesis. The pollen grains are mature at least a day before anthesis so that they may be transferred to the stigmas, but the anthers must be broken to liberate them.

RELATION OF AMOUNT OF POLLEN TO SHAPE OF FRUIT

To determine the amount of pollen necessary to bring about fruit development, pollen grains were collected on the point of a dis-

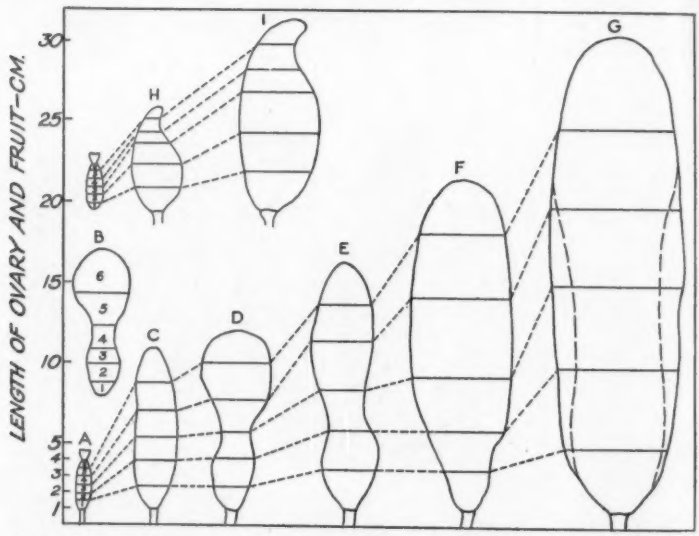

FIG. 4.—Comparison of growth of various parts of the ovary in fruits of different shapes. Seeds in fruits B and D to G are in the same stage of maturity. The difference in shape of the fruits is due to the time and duration of interruption in the development of the ovary

secting needle and counted under a dissecting binocular. A known number were placed on the stigmas of flowers which had been covered previous to anthesis. The pollinations were made after all developing fruits and fertilized ovaries were removed. The fruits were permitted to grow and the seeds were counted when mature. The plants varied considerably in vigor.

The results showed that in no case did the number of seeds exceed the number of pollen grains placed on the stigmas. The placing of pollen grains on one or all three of the lobes of the stigma did not change the location of the seed in the mature fruit. There was no correlation between the number of seeds produced and the shape of the fruit. A fruit with 2 seeds was as well shaped as one with 200 seeds, provided the fruit was grown on a vigorous vine. A large

number of nubbins shown in Figure 3, A, were produced on vines in poor vigor.

EFFECT OF DEVELOPING FRUITS ON THE SHAPE OF LATER-POLLINATED FRUITS

The detrimental effect of a developing fruit on later-pollinated flowers was indicated by such types as are shown in Figure 3, B, which when mature were stunted, with a premature yellowing of the shoulders. In order to determine the effect of dwarfing of the fruit on the subsequent growth of its various parts, small rubber bands were placed at equal distances on a number of small fruits, some of which were growing normally while others had had their growth cycles interrupted at various stages. Daily observations were made. Figure 4 shows the amount of growth produced in various parts of the small fruit in different types of fruit. The fruit are drawn to scale for comparison.

In Figure 4, A represents an ovary at anthesis, while B is a stunted fruit the ovary of which did not begin to grow until 22 days after pollination, when only the two sections in the tip developed seeds. C grew immediately after pollination and represents an ovary eight days after pollination, which is a critical stage for the embryos in the ovary. A period of inhibition[5] will mature many of the young embryos if prolonged for any length of time. The location of the matured embryos depends on the stage at which inhibition exerts its influence. An ovary eight days after pollination will begin to exert an inhibitory effect on later-pollinated flowers if the food in the plant becomes scarce. Figure 4, D, E, and F, shows the effect of stopping the growth of the ovary at the stage illustrated in Figure 4, C, for 15, 9, and 5 days, respectively. Maturity of the tissue begins at the base and increases toward the tip as the period of inhibition is prolonged. Figure 4, G, represents a normal fruit with a wasp type outlined to show this effect without a decrease in size. Fruits in Figure 4, B and D to G, have seeds at the same stage of maturity; H and I show the region of growth of two nubbins. In the nubbin the growth rate is retarded so that the ovules in the base of the small fruit develop at the expense of the ovules in the tip portion and there is a corresponding growth of the regions in the base of the small fruit instead of in the tip portion as is true in the wasp type of fruit. It is reasonable to suppose that ovules in all parts of the ovary become fertilized.

GROWTH PERIOD OF OVARIES AND FRUITS

Daily measurements were taken on a large number of developing fruits for length and diameter, to determine the variation of growth curves caused by external influences. These measurements were begun at anthesis and continued until maturity of the fruit. In Figure 5 the growth curves for fruits produced on typical, heavy-set plants are shown, together with the corresponding outlines of these fruits. In Figure 6, A, the main stem of this plant is shown with numbers indicating the location of the fruits corresponding to the numbers in Figure 5.

[5] In these studies inhibition is considered to be the effect whereby a maturing fruit prevents the growth of later-pollinated flowers.

At a glance it will be seen that the growth of the ovaries is interrupted at various periods after fertilization and that the production of fruit on the vine is divided into very definite periods. During each period two or three fruits matured, the removal of which gave an impetus of growth to a large number of ovaries and small fruits. The number of ovaries that develop normally depends on the amount of available food in the plant. A comparison of the shapes of fruits

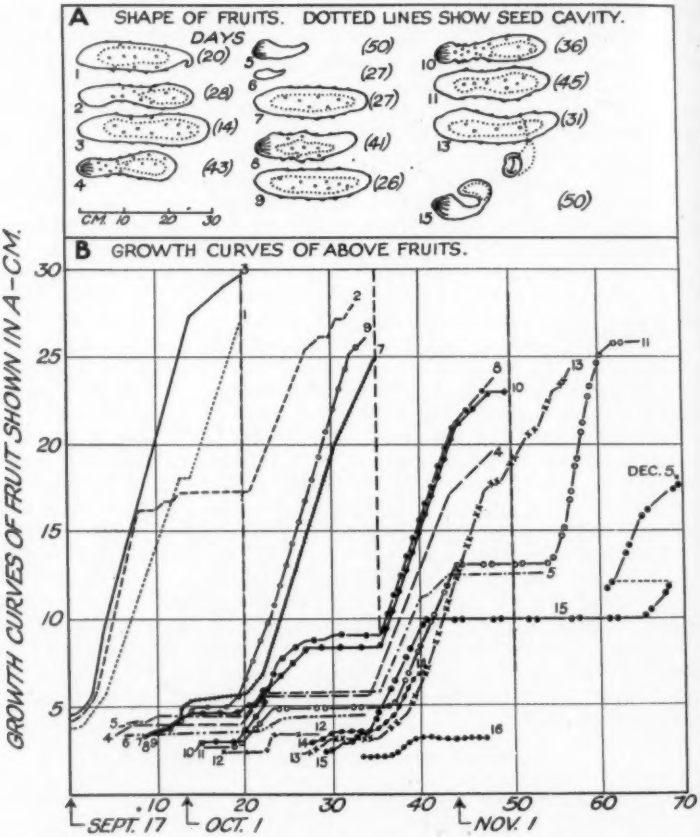

FIG. 5.—A, shape of fruits and number of days from pollination to maturity; B, growth curves for each of 16 fruits produced on one cucumber vine from September 17 to December 5

2, 4, 10, and 11 in Figure 5, with their respective growth curves, show that the wasp type may also result from an interruption in the growth cycle of the fruit.

An examination of the seeds showed that the ovules had partially developed in the constricted region of those fruits in which the interruption of the growth cycle of the fruit occurred five or more days after fertilization had been accomplished. Viable seed was found only in

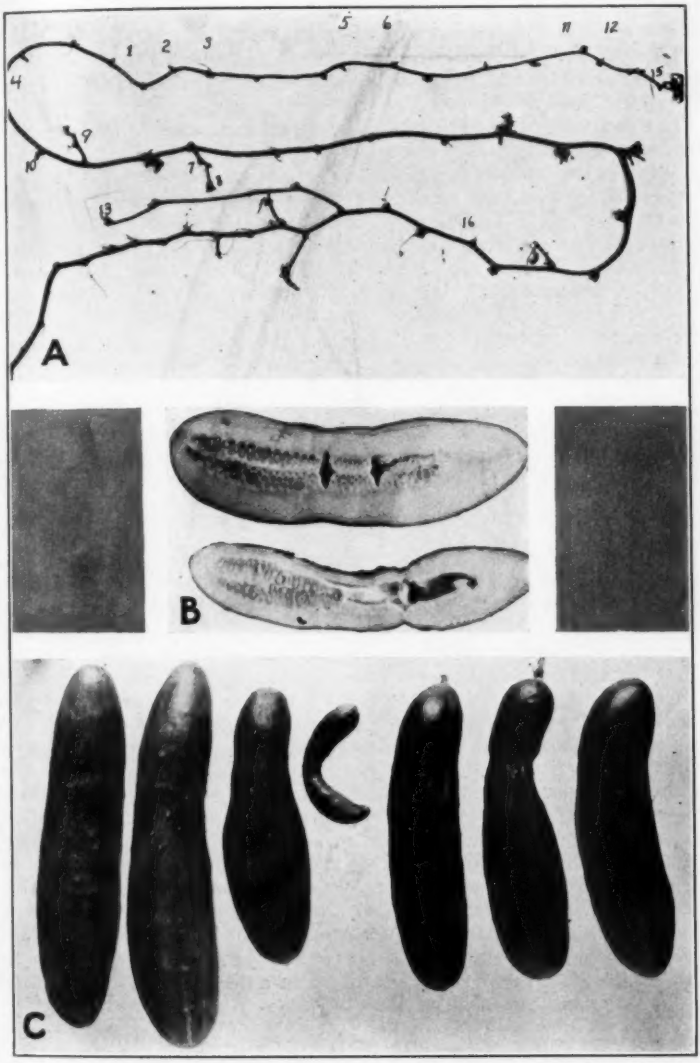

FIG. 6.—A, Main stem of cucumber plant the fruits of which are shown in Figure 5. The fruits and the nodes at which they were borne bear corresponding numbers. B, Fruits showing pithy and hollow centers caused by interruption in growth. C, Fruits in which the wasp shape is caused by inhibition of growth

the bulbous portion of the fruit. In fruit 10, Figure 5, which had been interrupted twice before reaching a length of 8 cm., a hollow pithy condition resulted, similar to that shown in Figure 6, B. No development of the embryos was visible in the constricted portion. The ovaries of fruits 4, 5, and 6 stopped growing before fertilization

could be accomplished. Fruit 4 developed the characteristic wasp shape, while 5 and 6 dried up before maturing any seeds. In Figure 6, C, a number of fruits from another vine are shown in which the wasping effect is due to the inhibition of growth. The effect on the size of the fruit is evident.

The location of the fruits on the vines has some relation to their final size and shape, as is shown in Figure 5. Fruits 7 and 9 located on laterals had an advantage in the second period over 4, 5, and 6 on the main stem. The prolonged interruption accounts for the inability of fruits 5 and 6 to recover from the period of inhibition. Number 7 had an advantage over 8 because the general flow of food from the main stem into the lateral reached 7 before it reached 8. Fruit 13, pollinated 10 days after 11 on the main stem, had an advantage because of its location on a lateral and apparently inhibited 11 from growing in the fourth fruiting period, even though it was a distance of 41 nodes away. General flow of plant food seems to be the only explanation for inhibition of growth and even this seems unsatisfactory.

Plants having more laterals and fewer pistillate flowers on the main stem do not show such contrasting results because fewer fruits develop at one time and there is a greater amount of leaf surface for each ovary.

EFFECT OF LIGHT ON THE DEVELOPMENT OF FRUIT

In Figure 7, A, are shown a number of fruits, on plants grown from December to February, subjected to different conditions of light. The plants from which the four large fruits in the top row were taken received electric light in addition to sunlight from two hours after midnight to sunrise; the four large fruits in the bottom row were taken from the control plants; and the six small fruits on the right were taken from plants shaded from 3 o'clock in the afternoon until 9 o'clock the following morning. Although the electric light (100 foot-candles) increased the size of the fruit and the number of seeds slightly, the greatest difference occurred in the shaded plants where fertilization of the ovules did not take place even though viable pollen was used. No seeds were produced in these fruits. The plants also were somewhat weaker than the controls. Although the shaded plants received sunlight during its greatest intensity for 6 hours out of a possible 10 hours of daylight, seed formation was entirely inhibited. Reducing the sunlight stimulated the production of seedless fruits on vines which would not produce fruit without fertilization during days of maximum sunlight. The parthenocarpic condition found in English varieties was induced in a nonparthenocarpic strain by changing the nutrition of the plant.

DISCUSSION

The data show that anything interfering with the development of the embryo affects the shape of the fruit, the degree of deviation from the normal depending on the number of ovules which fail to produce viable seed and the amount of tissue produced around the seed cavity. The growth of the seed in any portion of the ovary causes that portion to bulge or fill out. Uneven distribution of seed necessarily produces an irregularly shaped fruit.

LATE POLLINATION

Flowers pollinated one or two days after anthesis produce wasp-shaped fruits because only the ovules near the stigma produce seed. This may be due to some change in the nutrition of the ovary so that

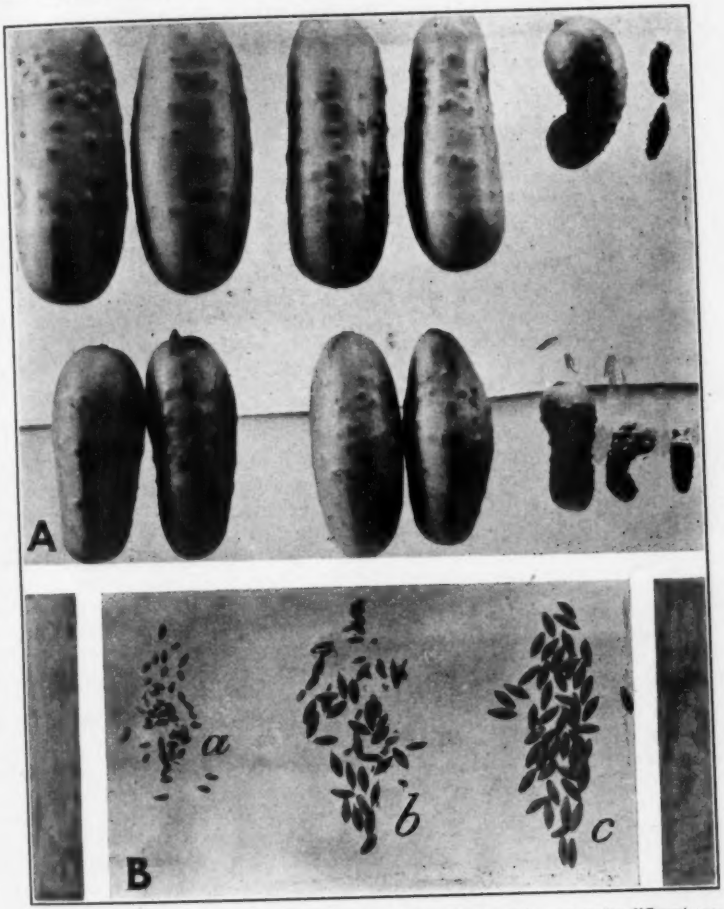

FIG. 7.—A, Cucumber fruits on plants grown from December to February under different conditions of light. The four large fruits in top row taken from plants receiving electric light in addition to sunlight from two hours after midnight to sunrise; the four large fruits in bottom row taken from control plants; the six small fruits on the right taken from plants shaded from 3 p. m. to 9 a. m. B, *a*, Cucumber seeds from a fruit developed parthenocarpically during days of minimum sunlight; *b*, seeds from a fruit interrupted in its growth; *c*, seeds from a normal fruit

the pollen tube finds it difficult to reach the ovules in the base, or to the fact that the ovules have passed the receptive stage. The latter probably depends on the nutritional condition in the ovary.

The degree of constriction depends on the amount of tissue that the plant forms around the seed cavity. Apparently the growth

of the various regions of the ovary is not interfered with, although seeds are produced only in a portion of the fruit. If late pollination is associated with inhibition of the growth period, then of course the length of the fruit will be reduced. If there is a surplus of food available for the growth of the fruit, the visible wasping effect may be completely obliterated even though the seeds develop in only a portion of the fruit.

INTERRUPTION OF THE GROWTH PERIOD

Flowers pollinated simultaneously at the same node develop as twins and are very uniform in size and shape, although they may not resemble the type characteristic for the plant. If a flower is pollinated one day earlier than another, it exerts an inhibitory effect on the flower subsequently pollinated when the supply of food in the plant is not sufficient to mature all the fertilized ovaries. This inhibitory effect is exerted as long as the fruit remains on the vine. If this period is not too long, the later-fertilized ovaries resume their growth. The whole phenomenon is correlated with the amount of food that is available in the plant at one time.

In flowers at a node pollinated simultaneously, the food is divided evenly among them, as is shown by the uniformity in size, shape, and the time of maturity of the fruit. In flowers pollinated at different times there is an uneven distribution of food, and the first-pollinated flower receives the advantage. The shape of the fruits resulting from this inhibitory influence shows a direct correlation between the length of the inhibitory period and the degree of constriction of the fruit. Fruit No. 15 shown in Figure 5 is a good example of the maximum time that the growth of the ovary may be inhibited without dying and yet produce some viable seeds. This flower was pollinated on October 28, grew very slowly for 7 days, grew normally for 7 days, was inhibited for 24 days, resumed growth, and matured five seeds on December 5. For 50 days the deformed fruit remained on the vine and matured seeds after being practically dead at the end of the period of inhibition. It was the only fruit on the vine after the tip resumed growth.

The effect of this inhibition on the shape of the fruit is evident and has considerable application to practices followed by the grower in producing fancy fruit. Whether the fruit is a nubbin (fig. 3, A) or whether it is a wasp (fig. 2, C) depends on the stage of development of the embryo when inhibition takes place. These fruits are practically opposite in type of development, due to the time at which inhibition exerted its influence.

When three flowers are pollinated simultaneously at different nodes there is usually sufficient food available for their development and normal growth. If the food becomes depleted to the point where one of the small fruits slows down in its growth the ovules in the base have developed sufficiently so that they continue to grow at the expense of those in the tip. Starvation gradually causes a dwarfing of the fruit. The food may be curtailed for the second fruit, but it will become much larger before dwarfing takes place. Curtailing the food supply results in nubbins of various sizes. Food apparently is directed into one ovary in preference to another. The location on the plant may determine their final size. In the opposite

type where wasping occurs there is an abrupt stop in the growth period. Can one say that the food is abruptly stopped in its flow to these fruits in favor of others? To do so might almost give the plant credit for directive growth of the ovaries. Were the slowing-up process gradual it might be attributed to starvation.

If food is suddenly made available by the removal of a developing fruit, a nubbin may resume growth and reach considerable size by an extension of the regions in the tip. In the wasp fruit, growth is resumed in the region where the seed is developed so that the fruit does not materially increase its length if the period of inhibition has been much prolonged.

An examination of a large number of these different abnormalities shows that in flowers pollinated at anthesis, if the embryos reach a certain size by uninterrupted growth immediately following fertilization, a period of inhibition of considerable duration will not prevent the larger ones from resuming growth. The larger embryos will be near the base of the ovary and account for the formation of nubbins. The two fruits on the left in the lower row in Figure 3, A, are characteristic of the type of fruit in which the period of inhibition was sufficiently prolonged so that the embryos in the tip did not resume growth.

If the period of inhibition follows immediately after pollination, fertilization of the ovules in the base of the ovary may not be accomplished. The flow of plant food into the ovary is stopped so that the pollen tube is unable to grow because of insufficient nutrients. Only the ovules in the tip close to the stigma become fertilized if any fertilization takes place. A prolonged period of inhibition may not prevent the embryos in the tip from developing when inhibition is removed.

The ovary increases in size very slowly the first few days after fertilization. If growth is not inhibited until after fertilization of the ovules, many probably become matured in the base of the ovary, because they do not resume growth after a period of inhibition. The seeds in Figure 7, B, *c*, were taken from a normal fruit, those in Figure 7, *b*, from a fruit which was interrupted in its growth, and those in Figure 7, *a*, from a fruit developed parthenocarpically during days of minimum sunlight. The sterile seeds in *b* show the effect of inhibition of the growth of the ovary after fertilization has taken place.

LIGHT CONDITIONS AND SHAPE OF FRUIT

During days of maximum sunlight a flower must be properly fertilized before growth of the ovary takes place. There is also less tendency for fruits to develop parthenocarpically. A fruit which has been inhibited in its growth rate will mature much quicker and produce very characteristic "yellow pickles." [6] As the amount of sunlight decreases in intensity and duration, there is a gradual change toward what might be termed a "vegetative" response. Less stimulation is necessary to start fruit development and many plants will produce parthenocarpic fruits. The yellow-pickle condition becomes less pronounced because of the prolonged life of the tissue in an inhibited fruit. Yellowing of the base of the fruit is much slower. The

[6] TIEDJENS, V. A. YELLOW PICKLE IN GREENHOUSE CUCUMBERS. Mass. Agr. Expt. Sta. Bul. 225, 8 p. 1925.

condition leads to more abnormally shaped fruits during days of reduced sunlight because ovaries which produce yellow pickles during days of maximum sunlight become sufficiently developed during days of minimum sunlight to be classed as seconds. Some of these are shown in Figure 3, B. There is a slight yellowing of the shoulders of small fruits due to maturity of the tissue before the seeds mature in the tip of the fruits.

The whole problem resolves itself into one of carbohydrate starvation. Reducing the light reduces the carbon compounds so that nitrogen metabolism may be modified. Apparently the surplus of sugar has much to do with the inhibition of parthenocarpic fruits during days of maximum sunlight. To say that tissues are matured more quickly by sufficient carbon for skeletal structures would be merely a hypothesis, but such seems to be the case.

RELATION OF SHAPE AND SIZE OF FRUIT TO GENETIC STUDIES

It has been shown that conditions of environment have a profound effect on the shape and size of mature fruits. Many studies in size inheritance involve the measurement of fruits for data indicative of the genetic potentiality of a plant. In Figure 6, C, are shown a few fruits of a single cucumber plant taken at different times in its life cycle. Inhibition in the growth of the fruits has had a tremendous effect on their shape and size.

The practice of taking data on size of the mature fruits of a plant to obtain an average or mean for the genetic potentialities seems inadequate. A big-fruited plant may be classed as intermediate because of environmental factors which reduce the size of its fruit. More reliable data would result if observations were taken from the maximum-sized fruit produced by the plant because these data would really indicate the potentialities of the plant for size of fruit. This could be accomplished by limiting the controlling factors to a minimum. Thinning fruit on the vine to the number that the plant will mature successfully would give much more reliable information than the present practice, even though the results were based on relatively few fruits.

Averaging of fruits for size makes it difficult to correlate size with other factors because size is variable. If a maximum-sized fruit for the plant is used, it becomes much less difficult to study linkage between size and other characters. As an example, the writer was interested in correlating size of fruit with certain vegetative characters. By using the maximum size of fruit for the plant very good linkage could be shown, while the average of all the fruits showed no linkage because the average for a plant was too close to the average for the entire population. This was especially true of the long-fruited selections. The longest fruit on one plant was 42 cm., while the average or mean length of its 27 fruits was 34.5 cm. This discrepancy in length was due to the various conditions discussed in this paper. The distribution curves for the short-fruited types are quite narrow, so that the mean is very close to the maximum size for the plant. Thus, maximum length seems to be the logical figure to use as typical for the cucumber fruit to expedite genetic research in size inheritance and its linkage relations to morphological characters.

CONCLUSIONS

Nubbins are fertilized ovaries gradually starved after their embryos have developed to a certain stage without absolute inhibition of growth. The cause of the "wasp," in addition to delayed pollination, is due to absolute inhibition of growth by prevention of fertilization or by maturation of the fertilized ovules before the embryo has reached sufficient size to recover. It is, then, a question of determining the relation of the inhibition phenomenon to starvation, which is not clear at the present time. Starvation seems to be a gradual process, while inhibition is abrupt. Both processes may be fundamentally the same.

From the results obtained by increasing and decreasing the light increment, the availability of carbohydrates in the plants seems to have a tremendous effect on the shape of the fruit and fertilization of the ovules. The surplus of sugars in the plant probably has some bearing on whether the fruit is gradually stopped in its growth period or whether it is abruptly inhibited. The data from the shaded plants suggest some relation between duration of periods of illumination and darkness. The six hours of illumination probably were so much out of proportion to the hours of darkness that the nutrient balance was completely upset.

In a study of size inheritance and its linkage relations to morphological characters in the cucumber, it is recommended that instead of averaging fruit for size, as is commonly done,the observations be taken from the maximum-sized fruit produced by the plant.

FROG-EYE LEAF SPOT OF SOY BEAN CAUSED BY CERCOSPORA DIAZU MIURA[1]

By SAMUEL G. LEHMAN

Plant Pathologist, North Carolina Agricultural Experiment Station

INTRODUCTION

The soy bean, *Soja max* (L.) Piper, is subject to a number of diseases which are manifest primarily in the form of leaf spots. Some of these which have somewhat recently been described are bacterial blight (*3*, *11*),[2] bacterial pustule (*5*, *12*), mildew (*7*), and brown spot (*13*). Lately another leaf-spot disease new to America has appeared on soy bean and is already widespread in the South. As a matter of convenience for reference, as well as appropriateness of symptoms, the name "frog-eye" will be used as a common designation of this disease. It is the purpose of the present paper to give a brief account of frog-eye leaf spot of soy bean and to describe the organism which causes it.

HISTORICAL ACCOUNT

Except for a few earlier and very brief statements (*14*) [3] regarding the occurrence of the disease herein described, there has appeared as yet in America no account of the active parasitic attack on soy bean by any fungus in the genus Cercospora. In 1901 Carver (*2*, *p. 5*), in publishing a list of 75 species of Cercosporae found in Macon County, Ala., noted the occurrence of a fungus, which he designated *Cercospora canescens* E. & M., on soy bean and several other unrelated hosts. This appears to be the earliest reported occurrence of a Cercospora on soy bean in America. The occurrence only was noted, however, and no description of the symptoms of the disease was given. The writer has not seen specimens of this collection, but it appears from Carver's description, as given in correspondence and from examination of similar specimens collected and sent to the writer during the summer of 1927, that *C. canescens* is saprophytic or at most only weakly parasitic on soy-bean leaves. Certainly the appearance of soy-bean leaves bearing *C. canescens* is symptomatically very unlike that of leaves attacked by the frog-eye disease. Likewise, the morphology of the organisms associated with the two diseases is very different.

In 1921 Miura (*9*) in a brief mycological note published a description of a Cercospora leaf-spot disease of soy bean which he had found at Tu-men-ling, south Manchuria, August 19, 1918. He ascribed it to a new fungus, which he named *Cercospora diazu*. The same disease was observed in Japan in 1924 by Hemmi, and diseased material was sent to F. A. Wolf, along with other specimens

[1] Received for publication Mar. 5, 1928; issued July, 1928. Published with the approval of the Director of the North Carolina Experiment Station as paper No. 25 of the journal series.

[2] Reference is made by number (italic) to "Literature cited," p. 832.

[3] HASKELL, R. J. DISEASES OF CEREAL AND FORAGE CROPS IN THE UNITED STATES IN 1925. U. S. Dept. Agr., Bur. Plant Indus., Plant Disease Rptr., Sup. 48: 376. 1926. [Mimeographed.]

of soy-bean diseases of the Orient. The North Carolina leaf spot was reported by Wolf and Lehman in 1926 (*14*) as identical with that found by Miura in Manchuria in 1918.

In 1924 Moore [4] recorded the occurrence of a Cercospora on soy bean in South Carolina. The disease was present on about 20 per cent of the plants in one field at Clemson College. No specimens of this collection are available for study, and the identity of the disease remains in question. It seems probable, however, as indicated below, that this may well have been the first reported occurrence of the frog-eye leaf spot of soy bean in America.

In 1925 Weimer [5] collected specimens of a soy-bean leaf spot at Clemson College and Orangeburg, S. C., Starkville, Miss., and Baton Rouge, La. In 1926 Moore again collected specimens of a Cercospora leaf spot on soy bean from a section of South Carolina remote from Clemson. Representative specimens of the 1925 collection by Weimer in South Carolina and the 1926 collection by Moore have been examined by the writer and identified as frog-eye.

Under date of August 15, 1925,[5] C. W. Edgerton reported the occurrence of a Cercospora leaf spot of soy bean in Louisiana and gave 1925 as the first year in which the disease had been observed in that State. Through the kindness of Edgerton the writer has been able to examine specimens of the Louisiana disease and regards it as identical with that found by Miura in Manchuria in 1918 and further described by the writer in the present paper.

Frog-eye of soy bean was first seen by the writer on September 23, 1925. At that time the disease was observed in a variety planting near Moyock, N. C. Lesions were very numerous on leaves of the Laredo and Otootan varieties. As far as the writer is aware, this observation constitutes the first knowledge of the presence of the frog-eye disease in North Carolina.

The brief historical account given above includes all reports of the occurrence of frog-eye on soy bean which have come to the writer's attention. It is evident, however, that more than one species of the genus Cercospora occurs on soy bean. This conclusion is supported by the writer's experience in September, 1923, when a hitherto unreported occurrence of a Cercospora on soy bean was observed in the experimental plots at Raleigh, N. C. The aspect of the disease was entirely different from that of frog-eye. The fungus had spread over rather large areas of the leaf and was not limited to definitely bordered spots. The leaf tissues were more or less moribund, a condition which may have preceded or may have been caused by the attack of the fungus. Although the fungus was examined microscopically, its species was not determined nor have specimens been preserved. Again in the summer of 1927 the writer observed a Cercospora fruiting on dead areas in living soy-bean leaves. In this case it appeared that the tissues had first been killed by confluence of lesions of the bacterial-pustule disease and that the Cercospora had come in as a secondary invader. Microscopic examination of this fungus showed it to be morphologically much like *C. canescens*.

[4] MELCHERS, L. E. DISEASES OF CEREAL AND FORAGE CROPS IN THE UNITED STATES IN 1924. U. S. Dept. Agr., Bur. Plant Indus., Plant Disease Rptr., Sup. 40: 186. 1925. [Mimeographed.]

[5] HASKELL, R. J. Op. cit.

In the summer of 1927 a lavender spot disease of soy-bean seeds was found in several fields in North Carolina. Although the writer has not been able as yet to determine definitely the identity of the causal organism, the trouble is symptomatically much like the disease described in Japan as due to *Cercospora kikuchii* Mat. & Tomo. (*8*). Experimental evidence thus far obtained indicates that the lavender seed-spot fungus in North Carolina is specifically different from that which causes frog-eye.

DESCRIPTION OF FROG-EYE LEAF SPOT

Frog-eye leaf spot is primarily a foliage disease. Lesions have never been observed as yet on pods, and in only one instance have they been found on stems. On leaves, diseased spots arising from single infection centers vary in size from mere specks up to 5 mm. in diameter. The spots are usually discrete, but occasionally several coalesce, forming diseased areas as large as 10 mm. in diameter. The sizes most commonly observed fall within the limits of 1 to 3 mm.

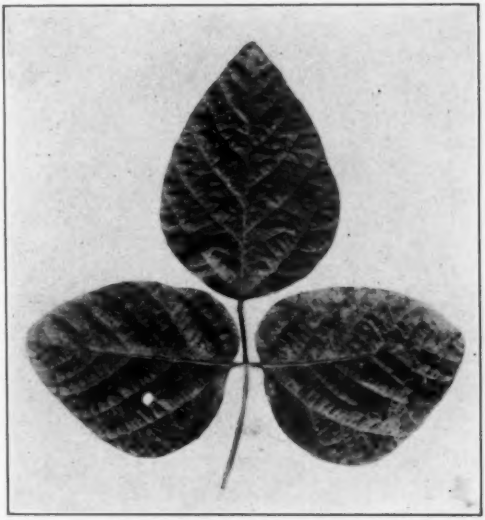

FIG. 1.—Very young lesions of frog-eye on a leaf of the Laredo variety of soy bean brought from the field September 27, 1927. The lesions are rather numerous on each leaflet, and the largest of them have begun to show the light centers. Actual length of the tip of leaflet 2½ inches

The symptoms in the early stages of infection vary somewhat with different varieties of soy bean and with leaves grown in a moist or dry environment. On leaves of the Laredo and Otootan varieties the lesions first appear on the upper side as minute reddish brown spots varying from 0.25 to 0.5 mm. in diameter. (Fig. 1.) At this stage the color on the lower side of the diseased leaf differs but little from that of normal leaf tissue, being only slightly reddish or merely a different shade of green.

By the time the diameter has increased to 0.8 mm. the reddish-brown color usually begins to fade from the center of the spot but remains at the margin of the lesions as a narrow reddish-brown border. (Fig. 2.) This color in turn passes abruptly into the green of the normal leaf without the intervention of a zone of chlorotic tissue. As the spot continues to enlarge, the reddish-brown band or border moves outward, always remaining very narrow and seldom exceeding 0.25 mm. in width. (Figs. 2, 3, and 4.) The central light-brown area enlarges apace, becoming still lighter, so that in the larger, fully developed spots the central diseased tissue is Hay's russet,

cinnamon buff, olive gray (*10*), or ash gray in color. At this stage the color of the diseased tissue is usually somewhat darker brown or darker gray on the lower side than on the upper side of the leaf and a given spot may show clearly a marginal dark-brown border on the upper side while this is not at all or only very faintly in evidence on the lower side. The disappearance of the green color and the development of the brown and gray shades go on more slowly on the lower side of the leaf than on the upper side. This difference in color is often enhanced by the presence of the fructifications of the causal fungus. Dark-colored conidiophore clusters develop on both sides of the leaf, but are usually fully twice as abundant on the lower as on the upper side. These fascicles of conidiophores are usually sufficiently prominent to constitute a definite sign by which the disease may be recognized unmistakably with the naked eye, or, at most, only a hand lens is needed to distinguish them from fructifications of other fungi parasitic on soy bean. They develop in the center of the lesion (fig. 5), and their dark color when massed obscures the light-brown or ash-gray color characteristic of the diseased tissues in the center of the spots, and thus the lower side of a lesion may appear upon cursory examination to be much darker than the upper side.

FIG. 2.—Fully developed lesions on a leaflet of the Laredo variety of soy bean brought from the field September 28, 1925. These lesions show light brown, ash gray, and almost white centers surrounded by a narrow reddish brown marginal border as they appear on the upper surface. This leaf is from the first collection of frog-eye in North Carolina

In older spots on which conidiophores have ceased spore production, the diseased tissue within the marginal brown ring becomes very thin, often paper white, and almost transparent. The dark-brown marginal ring is often raised above the general leaf surface, so that its thickness exceeds that of the adjacent normal leaf tissues.

In shape the spots arising from single loci of infection are roughly circular but irregular or angled spots are numerous (figs. 2, 3, and 4), the irregularity arising from growth difficulties when the causal organism encounters a veinlet. The spot develops in a uniform circular area within the areolae of the leaf, but whenever a veinlet is encountered growth is more rapid parallel to the veinlet than across it. Diseased areas arising from single loci of infection seldom if ever cross the midrib or the primary lateral veins of the leaf. Secondary lateral veins are frequently crossed, but even laterals of the third and fourth orders are traversed with some difficulty and usually give rise to more or less pronounced angularity of shape. Irregularity of shape is the rule when diseased areas arising from two or more loci of infection come in contact with each other. Because of the comparatively small size of the spots and the apparent inability of the causal fungus to destroy the vascular elements of the leaf, the diseased areas do not readily break up and fall out.

FIG 3.—Mature lesions on a leaflet brought from the field September 27, 1927. Note the high proportion of leaf area infected. Actual length of leaflet 3¼ inches

Frog-eye has been observed occurring naturally on stems in only one instance. This was in early October and on the variety Otootan. Nearly 100 per cent of the leaves were infected, each leaf bearing from few to many lesions. Notwithstanding this high leaf infection, cauline lesions were few in number and could be found only on the young, less woody portions of prostrate shaded stems.

These diseased areas have averaged two to three times as long as broad, the lesions lying in the direction of the long axis of the stem. On young lesions, one-fourth to one-half inch long, the central and oldest portion of the diseased area is English or mahogany red (*10*) and is surrounded by a zone of black, beyond which is the normal green of the young stem collenchyma. As the lesion grows older, it enlarges and the central area loses its red color, becoming first brown then a pale smoke gray. A persisting band of red separates the gray center from the outer bordering band of black. Minute stromata, often bearing fascicles of conidiophores with conidia, are present in numbers on the gray centers.

Careful isolations from the stem lesions described above yielded unmixed cultures of a Cercospora morphologically and culturally like strains previously isolated from leaves. Moreover, lesions produced on leaves in artificial inoculation tests with strains of the fungus isolated from stems were in no characters different from those produced by strains obtained from leaves.

Frog-eye is usually sufficiently well marked by characters peculiar to itself to be distinguished from other leaf spots of soy bean on the basis of symptoms and signs macroscopically visible. The brown specks representing the first stage of the disease resemble lesions produced by bacterial blight (*Bacterium glycineum* Coerper, *Bact. sojae* Wolf), but differ in that the latter are darker in color and decidedly more angular and are usually surrounded by a zone of chlorotic tissue, the last-mentioned symptom being entirely absent in frog-eye lesions. Frog-eye may be distinguished from bacterial pustule (*Bact. phaseoli* var. *sojense* Hedges) by the absence of the pustular outgrowths and chlorotic halos present in the early stages of the latter disease and by the absence of the uniform rusty-brown aspect characteristic of the later stages of bacterial pustule. In the case of brown spot (*Septoria glycines* Hemmi) of soy bean the spots enlarge up to 4 to 5 mm. but remain a uniform dark brown, reddish brown, or light brown according to the age and stage of pathogenesis and never develop spots with light-colored centers and reddish-brown borders characteristic of the frog-eye leaf spot. The mature spots of downy mildew (*Peronospora sojae* L. & W., *P. manshurica* (Naoumoff) Sydow in litt.) are symptomatically similar to advanced lesions of frog-eye, but the two diseases may be separated readily on the basis of the characters of their early developmental stages. A relatively large spot exhibiting mild chlorosis with no brown border represents the early stage of mildew; while in frog-eye lesions of this size the reddish-brown border is always present and the central tissues are dead, very light brown, ash gray, or even paper white. Mature

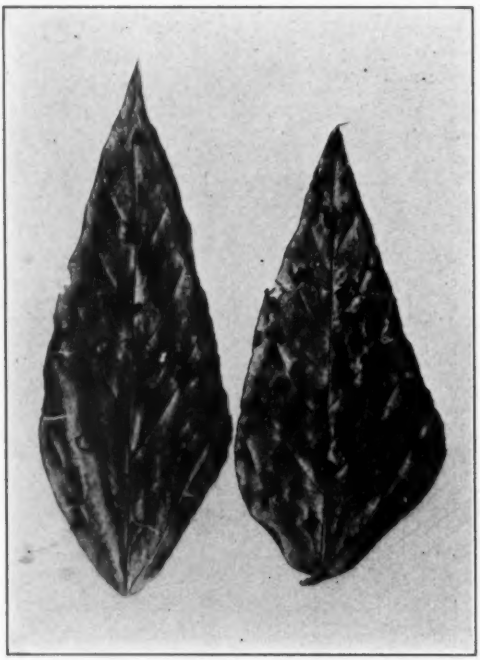

FIG. 4.—Mature lesions on leaflets of Otootan collected by W. D. Moore in South Carolina in 1926. About three-fourths natural size

spots of mildew are nearly always larger than frog-eye. Moreover, the mature spots may readily be separated on the basis of fructifications present on them. In mildew they occur only on the bottom side of the leaf and have a grayish or violet woolly aspect, while in the frog-eye disease the conidiophores occur on both surfaces of the diseased areas and have a brown to black aspect. These signs can usually be so easily recognized by the naked eye that the two diseases may be separated correctly.

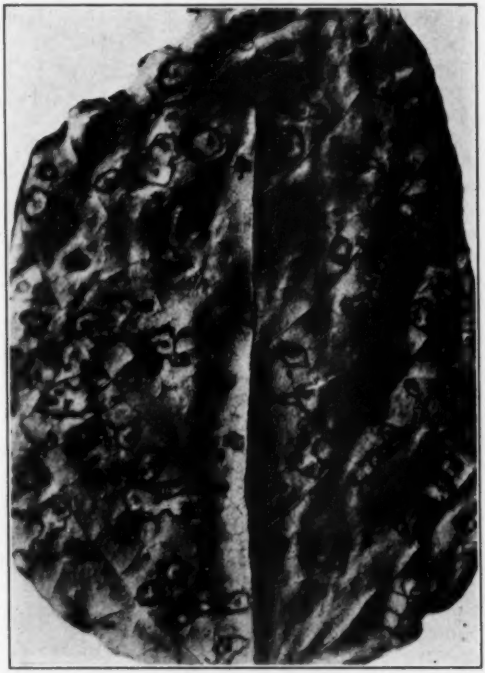

FIG. 5.—Portion of lower surface of diseased leaf enlarged 1.7 times in order to show more clearly the darkened central areas from which the conidiophore clusters arise. Note that the larger diseased areas are formed by coalescence of lesions arising from several points of infection

DISTRIBUTION OF THE DISEASE

Since the discovery of frog-eye at Moyock, Currituck County, N. C., the disease has been observed in a total of 12 counties. These 12 counties are widely distributed over the coastal plain and piedmont regions, the territory producing the greater part of the soy-bean crop of the State. (Fig. 6.) Outside of North Carolina the disease has been found in four States—namely, South Carolina, Georgia, Louisiana, and Mississippi. Correspondence with pathologists in all other of the

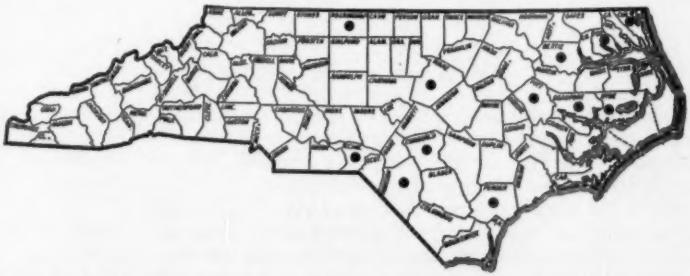

FIG. 6.—Map of North Carolina. The black dots indicate the counties in which frog-eye leaf spot of soy bean has been found

Southern States and in a number of Northern and Western States where soy beans are grown brought only negative reports regarding the occurrence of this disease. It is known to occur in Manchuria and Japan. These countries in all probability represent the region in which the disease originated and from which it spread to America.

ECONOMIC IMPORTANCE

In North Carolina the soy bean has taken rank as one of the main agricultural crops and is fast gaining in favor as a seed, forage, and soil-building crop throughout the South. In 1926 soy beans were grown on 159,000 acres in North Carolina and yielded seed valued at nearly $4,000,000. Other States are recognizing the value of this crop and are rapidly increasing the acreage devoted to it. It is difficult to estimate correctly the reduction in yield or value due to such a malady as Cercospora leaf spot. The fungus kills the tissues attacked and thus reduces the photosynthetic activity of the leaf and possibly also adversely affects the water relations of the plant, a condition which may attain significance in dry seasons. Ordinarily the infected areas do not involve more than 5 per cent of the leaf tissue of a plant, and in such cases the losses may not be great. However, plants are frequently seen on which 25 per cent or more of the total leaf area is diseased. The spots may be so numerous as to give the plants a yellowish aspect. In such cases reduction in yield must be considerable. The disease is potentially capable of doing great damage. It certainly has not been with us long, and possibly, with the greater accumulation of infective material, losses resulting from it may be considerably greater than at present. This is particularly true since the varieties which appear to be most susceptible are now rapidly coming into favor.

VARIETIES ATTACKED

The disease has been observed on the following varieties: Laredo, Otootan, Biloxi, Manchu, Mammoth Yellow, Goshen Prolific, Virginia, Austin, Tarheel Black, Wilson, Tokyo, Haberlandt, and Chiquita. Of this group, Otootan and Biloxi are most susceptible; Chiquita, Tarheel Black, Wilson, and Mammoth Yellow are somewhat less susceptible. Early maturing varieties such as Dixie, Manchu, and Virginia escape serious injury; while such late maturing varieties as Otootan and Biloxi suffer most.

ETIOLOGY

ISOLATION OF THE PATHOGENE

Diseased areas which have developed to maturity bear numerous conidiophores, each of which produces one to several conidia. Isolations are easily accomplished by the following method: Conidia scraped from the surface of a lesion are mounted in a drop of sterile water so as to make a dilute suspension. A loopful of this suspension is spread over the surface of a hard plain agar plate with a zigzag stroke. The spores germinate readily and in the course of five to six days thin gray colonies may be located and positively identified microscopically by observation of the conidia and conidiophores. If the isolations are made on plain agar from lesions on fresh, green

leaves, and the spore suspension is diluted, little difficulty is experienced in obtaining discrete colonies free from contaminating bacteria or fungi. Transfers may then be made from these colonies to other plates or to tubes.

DESCRIPTION OF THE CAUSAL FUNGUS

The fungus which causes frog-eye of soy bean sporulates abundantly by the formation of conidia and conidiophores on mature lesions. The latter occur in tufts or fascicles on both surfaces of the lesion but are present in greater numbers on the lower, less exposed side. The conidiophores composing a fascicle vary in number from 2 or 3 to as many as 25 and appear to arise from a stroma. (Fig. 7, A, B, C.) The stromata are always small and inconspicuous and often consist of little more than the aggregated basal cells of the conidiophores, but at other times there is evident development of stromal tissue. The stromata appear to arise on, in, or just beneath the epidermis and penetrate the leaf mesophyll to a depth equal to one-eighth to one-fourth the thickness of the leaf. Conidiophores viewed under apocromatic objectives are light brown (cinnamon) to dark brown (walnut brown) in color. They vary in length with favorable or unfavorable moisture conditions. Sizes ranging from 52 to 120 microns by 4 to 5.5 microns have been observed on specimens gathered from the field. They are usually 1 to 3 or 4 septate and show prominent geniculations and spore scars. (Fig. 8, H, I, J.) Each conidium is formed at the tip of the conidiophore (fig. 9, A, *a*, *b*), but the latter continues to elongate by pushing out at an angle from the original direction of growth. Thus it forms a kneelike flexure, grows forward (fig. 9, A, *b*, *c*; D, *d*, *e*, *f*) and produces another spore. When the spore falls away a scar is left at its place of attachment. (Fig. 9, D.) As many as 11 spore scars have been counted on a single conidiophore, the usual number being 1, 2, or 3. (Fig. 8, H, I, J.)

The conidia are multiseptate and hyaline, except that a few which have become light brown may be found at times on lesions which are several months old. They are elongate, fusiform, or tapering mostly toward the apex and less or not at all toward the base. The apex is rounded and about half as thick as the broadest part of the spore. The base is usually rounded and bears a circular scar marking the place of attachment to the sporophore. The septa vary in number from 0 to 10. The 3-septate forms are most numerous, 33 to 53 per cent of the spores falling into this group. The classes represented by 4, 5, 6, and 7 septate forms are approximately equal in point of numbers, each of these classes containing about one-fourth to one-half as many spores as the 3-septate group, or about 10 per cent of the total number of spores. On leaves the conidia range in size from 24 to 108 microns by 3 to 9 microns, the most common sizes being 40 to 60 microns by 6 to 8 microns.

Table 1 shows the distribution of the conidia according to the septation classes in which they happen to fall, and Table 2 shows the average spore sizes in the different septation classes. When grown in culture on plain agar, the 3-septate forms remain by far the most numerous. In culture, however, the average sizes of all the septation classes are larger than those occurring in nature. Spore measurements taken from plain agar cultures of a Cercospora isolated from

cowpea show an entirely different distribution in septation classes, and the spore sizes are smaller than for the soy-bean Cercospora.

Conidia collected at different times and places and on different varieties of soy bean are shown in the camera lucida drawings of Figure 8, A–G. Spores from the variety Biloxi drawn seven days after the leaves were collected are shown in Figure 8, A. Most of the spores were still turgid, but in many of them the protoplasts

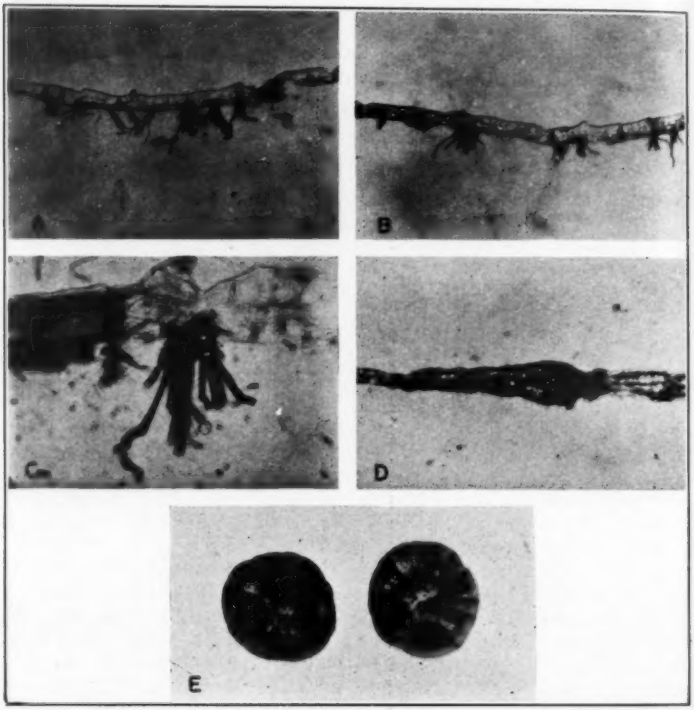

FIG. 7.—*Cercospora diazu* as it appears on lesions on soy bean leaves and in culture

A.—Paraffin section through the sporiferous area of a lesion. Note the clusters of conidiophores arising from the lower surface.

B.—Similar to A.

C.—A single conidiophore cluster enlarged to show septation and spore scars. Most of the conidiophores have broken off, but one with several spore scars is still intact.

D.—Paraffin section showing hypertrophied tissue in the region of the brown margin of the lesions. Undiseased tissue of normal thickness at right of the deeply staining, hypertrophied region; to the left is the direction of the center of the lesion.

E.—Colonies of *Cercospora diazu* growing on potato-agar plates incubated 15 days at 25° C.

in one to four cells had disintegrated, allowing partial collapse of the walls. The spores of Figure 8, B and C, were taken from different types of lesions occurring on the same collection of leaves of the Otootan variety, those of B being from a larger, rather young, greenish brown spot with a dark brown border and those of C from an older spot with the usual dark brown border and ash-gray or white center. It was at first thought that these two types of lesions might be caused by two different species of Cercospora, but the similarity

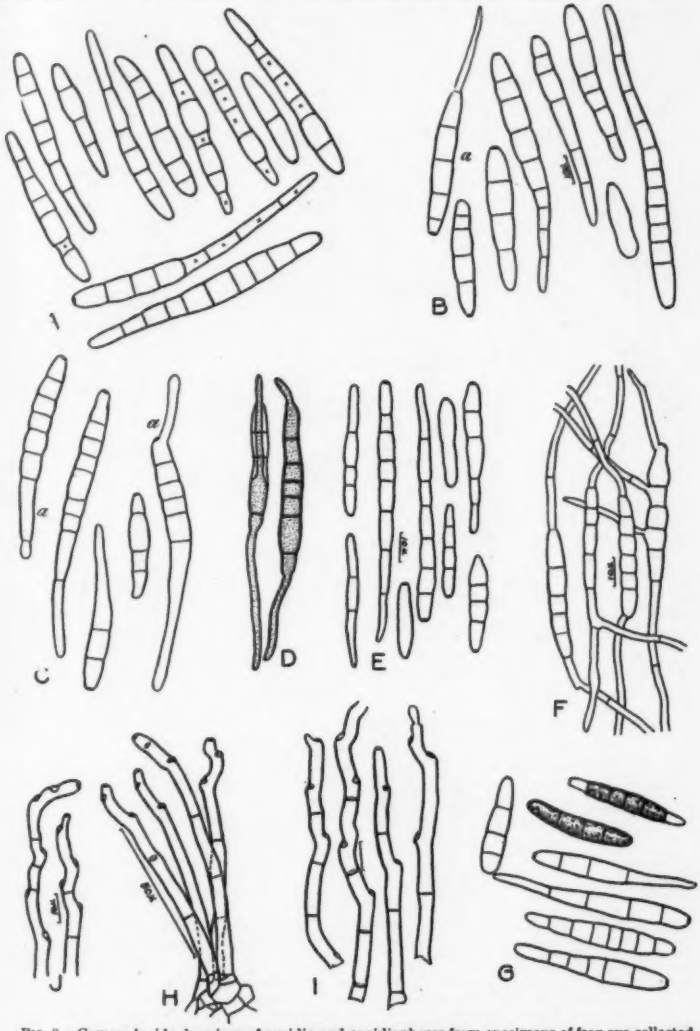

FIG. 8.—Camera lucida drawings of conidia and conidiophores from specimens of frog-eye collected at different times and places

A.—Conidia from leaves of the Biloxi variety of soy bean collected in Pitt County, September 21, 1926. Drawn seven days after date of collection. The cells marked *x* had lost turgidity following disintegration of their protoplasts. Approximately 25 per cent of the conidia of this collection were in this condition. The remaining cells were turgid and capable of germination.

B, C.—Conidia from leaves of the Otootan variety collected in Perquimans County, September 2, 1926, those of B coming from a large rather young greenish-brown spot with dark-brown border, and those of C from an older spot with the usual ash-gray or white center and dark-brown border. Conidia marked *a* were germinating when drawn.

D.—Germinating conidia scraped from a leaf 81 days after date of collection and 24 hours after the air-dry leaves had been placed in a moist chamber.

E.—Conidia from leaves of the Otootan variety. Collected October 1, 1925, from the field in Currituck County, in which the disease was originally found in North Carolina.

F.—Conidia of E germinating after 18 hours in tap water.

G.—Conidia from specimens of frog-eye received from Japan.

H.—A cluster of conidiophores from leaf of the Biloxi variety.

I, J.—Conidiophores from leaves of the Otootan variety of different collections, I coming from the collection noted under B and C above, and J from collection noted under E.

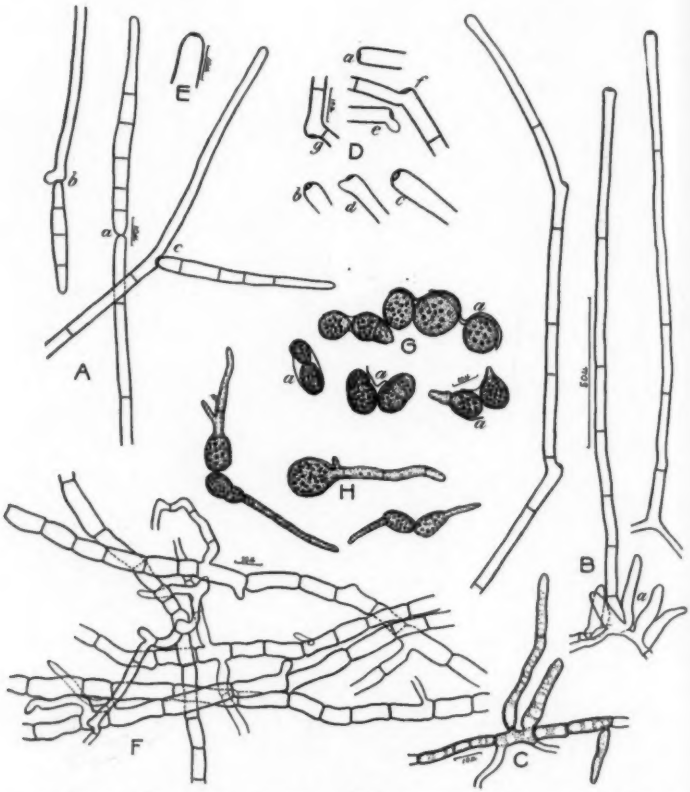

FIG. 9.—Camera lucida drawings showing morphological features of *Cercospora diazu* grown on culture media

A.—Conidiophores drawn with conidia in position: *a*, first conidium fully developed; *b*, a conidiophore beginning proliferation to form a second conidium; *c*, further development.

B.—Conidiophores grown on plain agar. These show numerous septations, prominent geniculations, and brown color. The short hyphae at *a* are young conidiophores. They have already turned brown, while the hyphae from which they arise are still hyaline.

C.—Young brown conidiophores arising from a hyaline hypha.

D.—Higher magnification showing terminal spore scars at *a*, *b*, *c*; continuation of growth beyond the spore scar at *d* and *e*; and geniculations with spore scars at *f* and *g*.

E.—Basal end of a spore showing scar at place of attachment to conidiophore.

F.—Mycelium of a young culture on a synthetic agar medium. Main hyphae are dilute brown, secondary hyphae hyaline.

G.—Swollen hyphal cells, "endospores," characteristic of the older mycelium produced on potato agar having a hydrogen-ion concentration of P_H 2.6. The protoplasts have swelled and ruptured the mother-cell wall and have formed new investing membranes. Vestiges of the mother-cell wall are visible at *a*.

H.—Cells of the type shown in G, germinating after 18 hours in tap water.

of the conidia produced on the two kinds of lesions does not warrant such an assumption. The spores of Figure 8, E, were also from Otootan leaves but from a collection of the previous year in a different part of the State. This collection came from the field in which the first specimens of frog-eye were found in North Carolina but the leaves were gathered 12 days later, at which time the lesions were old and no longer sporulating readily. The spores of this one collection averaged one to two microns narrower than those of collections made at other times and places, and might be considered by some students to represent a different species of Cercospora. However, since they are about the same in length as spores found on other collections and the lesions on which they occurred were not different from those of other collections, these spores are not herein treated as representing a separate species. The spores illustrated in Figure 8, G, were obtained from leaves furnished by T. Hemmi. These leaves had been collected in Japan in 1924 and the fungus on them had been identified by Hemmi as *Cercospora diazu*. The conidia on this material were few in number and somewhat shorter than those found on the frog-eye lesions so far collected in North Carolina. However, in diameter, shape, septation, and general appearance the conidia on the Japanese material are much like those found on the North Carolina collections and are here considered to represent the same species. It is generally believed that moisture conditions greatly influence dimensions of the conidia and conidiophores in the genus Cercospora. In the present study when pure-culture inoculations were made on soy-bean plants growing in glass-sided incubators where moisture was relatively abundant, both conidiophores and conidia, formed on the lesions which developed, were considerably longer and somewhat slenderer than those found on material collected in the field. (Table 2.) In this respect they were more like conidia and conidiophores grown in culture. Thus it seems that the difference noted above in respect to size of conidia on different collections may be due to growth under different moisture conditions.

TABLE 1.—*Conidia of Cercosporae from soy bean and cowpea distributed in classes on the basis of number of septa per conidium*

Source of conidia	Date and place of collection	Number of conidia in each septation class									Total number of conidia
		1 septate	2 septate	3 septate	4 septate	5 septate	6 septate	7 septate	8 septate	9 septate	
Otootan	October, 1925, Currituck County.	9	11	38	13	15	12	14	0	1	113
Mammoth Yellow	September, 1926, Pitt County.	7	5	44	12	13	13	9	4	2	109
Biloxi	September, 1926, Pitt County.	3	3	33	7	12	8	8	0	1	75
8-day-old culture of C. diazu on plain agar.		3	4	39	11	6	5	2	2	1	73
21-day-old culture of a cowpea Cercospora on potato agar.		1	3	18	15	24	24	14	3		102

TABLE 2.—*Average sizes of the conidia falling in the different septation classes shown in Table 1*

Source of conidia	Average size of conidia in each septation class								
	1 septate	2 septate	3 septate	4 septate	5 septate	6 septate	7 septate	8 septate	9 septate
Otootan, 1925, Currituck County.	33.7×4.1	39.8×4.6	43.2×5.3	57.6×5.5	61.2×6.7	64.6×7.1	76.7×6.6	.....	88×8
Mammoth Yellow, 1926, Pitt County.	37.7×6.3	38.4×6.8	42.1×7.2	51.4×7.6	52 ×7.4	59.3×7.6	66.6×7.6	78.5×8.2	88×8.3
Biloxi, 1926, Pitt County.	38 ×5.3	32.3×7	45.8×7.0	48.8×7.0	55.8×6.6	59.2×7.3	67.7×7.7	.....	.....
8-day-old culture of *C. diazu* on plain agar.	36.6×5.2	79.5×6.2	69.3×7.1	79.1×7.1	80.6×7.6	87.4×7.7	108 ×7.4	92.5×7.8	.....
21-day-old culture of a cowpea Cercospora on potato agar.	.....	42.0×8.2	49.0×7.5	57.2×7.8	61 ×7.8	68.2×7.9	73.8×7.8	77.3×7.7	.....

GERMINATION OF THE CONIDIA

The conidia germinate readily in tap water by sending out slender hyaline hyphae. (Fig. 8, D, F.) The process begins at favorable temperatures within an hour or so, and by the end of 18 hours long hyphae have been produced. Usually the end cells of a given conidium germinate first, but germ tubes may arise from one or more intervening cells of the same spore. At times two germ tubes arise from a single cell. In cultures conidia often germinate in situ, and have been observed to form a short germ tube which in turn produced a secondary conidium of normal size while the whole was still attached to the conidiophore.

Conidia are still able to germinate after a considerable time in a dry condition. Leaves of the Biloxi variety of soy bean were collected on September 24, brought into the laboratory, and stored in a dry condition in envelopes. On December 2, 69 days after the date of collection, conidia from lesions on these leaves were put in tap water to germinate. After 24 hours at 25° C. approximately 1 per cent of the conidia had germinated, sending out long phyhae. In a similar test conidia from Otootan leaves, which had been collected on August 30 and kept dry in the laboratory for 94 days, showed somewhat less than 1 per cent of germination after 24 hours at 25°. Spores which did germinate produced long vigorous germ tubes. The protoplasm in those spores which failed to germinate had lost all semblance of organization.

Dry leaves from the collections used in the above tests were at the same time placed in the damp atmosphere of a moist chamber. Twenty-four hours later spores were scraped from lesions on these leaves and about 1 per cent were found to have produced short germ tubes. (Fig. 9, D.) Spores from the same packet of leaves had shown nearly 100 per cent germination when tested after 24 days of storage.

Not all the cells of a multiseptate conidium lose viability at the same time. In old spores some of the cells appear to be entirely empty and are much shrunken, while in adjacent cells the protoplasts not only retain their usual finely granular structure but often appear to be more dense than in young conidia. It is not impossible

that this increased density is due to an absorption of protoplasmic materials from the cells which become empty by the cells which remain viable. Moreover, the longevity of the latter cells may be conditioned on this absorption.

INOCULATION

A suspension of conidia was obtained by macerating diseased tissue in water. This suspension of conidia and particles of diseased tissue was rubbed onto leaves of Laredo and Mammoth Yellow soy beans growing in the greenhouse. The Laredo plants were young and bore two to three trifoliate leaves; the Mammoth Yellow plants were older and carried many leaves. Inoculations were made in September 2, and the plants were kept under shaded bell jars in the greenhouse for two days thereafter. On September 12, 10 days after the inoculations, small red spots, the first evidence of infection, were found on Laredo, none being observed on Mammoth Yellow at this time. By September 20 the spots on Laredo had become typical frog-eye lesions and bore conidiophores and conidia. Several lesions were also found at this time on Mammoth Yellow leaves. Two days later these bore sporophores and conidia of the frog-eye fungus.

Another inoculation test was made using conidia from pure cultures. The conidia were obtained by gently washing the surface of a 92-day-old culture with tap water. This culture was growing on potato agar in a Petri dish kept in a moist chamber dish in the open laboratory since its inoculation. The colony had by this time reached 1 cm. of the edge of the dish and was producing conidia abundantly at its margins. The conidia were atomized onto soy beans of the Mammoth Yellow variety which had been growing for 22 days in glass-sided incubators at a temperature ranging from 18 to 24° C., but remaining for the greater portion of the time at 18°. At the time of inoculation the plants were tall (averaging 30 cm.) and bore three or four leaves each, but spindling owning to growth in reduced light. The leaves were thinner than normal apparently because of the reduced light and rather high humidity of the culture chambers. They were also smaller than normal for field-grown plants, but possessed a very good color and appeared healthy in every respect.

During the period of infection and development of the disease the temperature of the plant chamber ranged from 18° C. (at night) to 25° (in the afternoons) and averaged about 22°. There was no visible evidence of infection 5 days after inoculation, but at the time of the next observation, 11 days after inoculation, many red-bordered spots were in evidence on the leaves, the largest being about 1.5 mm. and the smallest about 0.5 mm. across. (Fig. 10.) The largest of these lesions must have been evident 8 days after inoculation. On the thirteenth day after inoculation some of the spots had produced sporophores and conidia typical of the fungus used in making the inoculations; also at this time, when the oldest spots were forming their first conidia, young new lesions were appearing in considerable numbers. Obviously the later-appearing lesions arose from conidia which had encountered some difficulty in establishing parasitic relationship with the host. Not only did conidia and conidiophores develop on older lesions but the fungus was recovered in pure culture by planting leaf tissue from young lesions which had shown no fruiting structures. It is judged that the period of incubation for this

disease under favorable moisture conditions and an average daily temperature of about 22° varies from 8 to 14 days.

Several other successful inoculation tests were made, but these need not be reported in detail. Disease lesions resulting from the inoculations made in the greenhouse were comparatively few in number, but they were typical of frog-eye symptomatically and bore fruiting structures identical in every way with those found so abundantly on lesions in the field. Apparently the environmental conditions maintained about the plants in the greenhouse inoculation tests were not so favorable for infection as those which occur at times in the field. Greater numbers of lesions developed in the test in the glass-sided plant chambers where humidity was higher and the plants more tender and growing rapidly. Nearly all the infections developed on leaves which were not yet full grown at the time of inoculation, very few lesions appearing at any time on the older and lower leaves. In a test in which young and old plants were inoculated at the same time from the same culture of the fungus, by far the greater number of lesions developed on the young plants, although these bore fewer leaves with much less total leaf area exposed to infection. Moreover, the average size of mature lesions occurring on young leaves is considerably greater than that of lesions produced on old leaves under the same conditions. Young leaves are not only more susceptible to infection but the fungus grows more rapidly in their tissues. Spores which do not immediately bring about infection of more mature leaves under conditions favorable for development of the disease on young plants sometimes live for several weeks on leaves. Then when conditions are made highly favorable as by covering the plants for a week with a bell jar, these spores grow and produce frog-eye lesions.

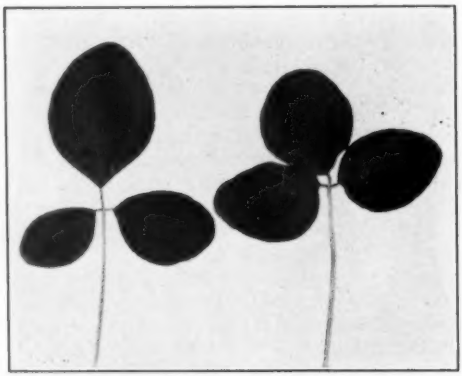

FIG. 10.—Lesions resulting from artificial inoculation of plants of the Mammoth Yellow variety of soy bean. The leaves are smaller and thinner than normal as a result of growth in glass-sided chambers under conditions of rather high humidity and reduced light

IDENTITY OF THE CAUSAL FUNGUS

The frog-eye disease of soy bean herein described is in all its major aspects like the leaf-spot disease which Miura (*9*) found in south Manchuria in 1918 and to which he ascribed, as causal organism, the new fungus *Cercospora diazu*. Likewise, the fungus found by the writer to be associated with frog-eye is morphologically very like that described by Miura. Miura's statement of the symptoms of the disease is very brief. Specimens sent from Japan by Hemmi and from Shizuoka by Nakata have been compared with material

collected in North Carolina, South Carolina, Georgia, and Louisiana and found to be identical in symptomatology and in the morphology of the causal organism. In order that it may be more readily accessible to American pathologists and mycologists, Miura's description is given herewith.

Cercospora diazu M. Miura, n. sp.

Amphigenous, spots at first dark brown, later pale brown at centre, irregular or circular in outline, solitary, rarely confluent, with dark colored border, 3–6 mm. across; conidiophores pale sooty colored, springing from a small stroma composed of a few cells, nonseptate or very rarely septate, 100–130 x 5; conidiospores hyaline, cylindrical or fusiform, apex rounded and the base somewhat acute, more or less thicker walled, 0–6 septate, not constricted at septa, acrogenous, 39–70 x 5–7.

On leaf of *Glycine hispida* Maxim. and *Glycine soja* S. et Z. Tu-men-ling, South Manchuria, August 19, 1918., leg. M. Miura.

The writer has observed certain minor details in which the fungus found in the United States differs from the description given by Miura. The vast majority of the conidia are hyaline, but an occasional sooty-colored spore is found on material kept dry in the laboratory for a few months. These dark-colored conidia have never been observed on leaves fresh from the field. Miura describes the conidia as cylindrical or fusiform and as having a rounded apex and somewhat acute base. As the writer has observed the conidia their greatest thickness is a measure from the base equal to one-fourth or one-third their total length and they taper much more strongly toward their apexes than toward their bases. (Fig. 8, A, B, C, E, and G.) The apexes are usually rounded but are sometimes long and acute. This was observed not only on American specimens but also on material received from Japan. It may be that the acute tip ends of certain of these conidia are the result of prolonged growth somewhat akin to germination. Again, the bases of the conidia can not be described as acute as indicated by Miura, for they are broader and more rounded than the apexes. The writer has also observed a greater range of conidial septation than that given by Miura, and also a greater range in size. However, this is probably merely the result of the observation of a greater number of conidia. Another point of difference in the material observed by the writer is found in the septation of the conidiophores. Septa quite commonly appear in the conidiophores, often occurring between successive geniculations (fig. 8, H, J, and fig. 9, B), but more commonly below the first spore scar (fig. 8, I). This is in marked contrast to the idea conveyed by Miura's words "nonseptate or very rarely septate." On the other hand, the lengths of the conidiophores observed by the writer are not so great as those reported by Miura.

The morphological differences noted above might be used by some students to establish a new species. It appears to the writer, however, that Miura's description of *Cercospora diazu* was based on a rather limited amount of material. Because of agreement in the broad morphological characters of the causal organisms and the very great similarity of the symptom complex resulting from parasitism of the common host, the writer prefers to consider the causal organisms associated with frog-eye as identical with *C. diazu*.

Cercospora glycines Cooke (*4, p. 39*) has been described as the cause of a leaf spot of *Glycines clandestinae* in Australia. This fungus is in

all probability not identical with *C. diazu* on soy bean. The two fungi not only occur on different host species, but differ also in the shape, size, color, and septation of their conidia.

PATHOLOGICAL ANATOMY

The exact method of infection, whether through stomatal apertures or by direct penetration of epidermal cells, has not been determined. The germination of conidia sown on leaves in moist chambers has been observed. Germ tubes which develop into long branching hyphae and form a sparse surface mycelium grow out from the conidia. These hyphae have been observed to grow directly across open stomata, but in no instance has it been possible to find a hypha entering the leaf through the stomatal aperture, nor even by direct penetration of the epidermal cells. It is thought that penetration may occur only after the hyphae have grown superficially for some two to four days and killed the epidermal cells by secretion of some toxic substance. Microtome sections of leaves fixed at the time the lesions first become visible show that the epidermal cells have completely collapsed over the entire area of the lesion and in some instances for a short distance beyond the marginal limit of dead mesophyll cells.

Mycelium is not at all abundant in diseased tissues. One usually finds not more than one to three short pieces of hyphae in a stained median section through a lesion. Even in the immediate vicinity of the minute stromata from which the conidiophores arise hyphae are difficult to find. Only a small proportion of the cells of the diseased area are in direct contact with hyphae of the parasite. The fungus apparently secretes some substance which by diffusion passes beyond the frontier of hyphal penetration and injures the host cells. Hyphae may be found near the margins of lesions, but always there is present the reaction indicating injury some two or more cells beyond the most advanced fungus filaments.

The first visible cytological change in injured cells as observed at the margins of sectioned lesions is a slight disintegration of the chloroplasts, accompanied by a change of staining reaction. Usually this is quickly followed by disorganization of the protoplast, loss of turgidity, and eventually by complete collapse of the cell. Apparently much cell-wall material is consumed, for only fragments of the cellulose walls remain in the older portions of the lesions. Epidermal cells are less resistant to toxic action of the fungus and always show complete collapse before the underlying cell protoplasts have lost turgidity. The older diseased areas are shrunken and much thinner than healthy tissue.

As the lesions increase in size the margin is marked by a reddish-brown band of varying width. This band indicates the region in which the cell protoplasts are undergoing disintegration. When the disease has run its course and the infected areas have reached full size, this marginal reddish band of tissue is often found to be thicker than that of adjacent undiseased tissue. (Fig. 7, D.) This thickening of the leaf tissue is due to hypertrophy of the cells delimiting the diseased area. By this time the fungus is exhausting itself in sporulation, and possibly the hypertrophy of host cells is due to stimulation resulting from the presence of very minute traces of the same toxic

material which when present in larger quantities causes cytolysis and death.

Diseased cells which are showing the early disintegration phenomena described above and also those which show hypertrophy always stain more deeply and are much less easily destained than either healthy cells or cells in the older portions of the lesions. The reddish-brown border marks the region of most deeply staining cells.

CULTURAL CHARACTERS

Cercospora diazu grows well on a variety of culture media in common laboratory use. Unlike many Cercosporae, this species sporulates plentifully on all media which support normal even though very sparse growth.

On plain agar (20 gm. chopped agar shreds in 1,000 c. c. of water) thin gray colonies form with a sparse development of aerial hyphae. The colonies are plainly evident at the end of five days and attain a diameter of 8 to 12 mm. in eight days. At this time long smoky-brown conidiophores bearing spores are present. The conidiophores become smoky brown even before forming their initial conidia (fig. 9, B, C), while the conidia and young feeding hyphae remain hyaline. Older hyphae (fig. 9, F) and more mature conidiophores are darker brown and give a gray to brown aspect to the colony, the color becoming darker as the number of conidiophores increases. Plain agar is well suited as a medium on which to study conidiophore development. Growth is not so dense but that individual hyphae can be traced for long distances and conidiophore primordia (fig. 9, B, A, C) easily found.

On potato-dextrose agar [6] the colonies develop as compact mycelial mats with velvet surfaces formed by close stands of vertically growing hyphae. By the time the colony has reached a diameter of 20 to 25 mm. its surface is marked by convolutions or folds disposed in both radial and concentric fashion. (Fig. 7, E.) The radial ridges, some 4 to 10 in number, are the more prominent, the circular folds being plainly evident but less pronounced. The surface of the colony is not only characterized by these physiographic features but is also marked by concentrically arranged color bands. The outermost edge of the colony where the young hyphal tips are invading the medium is a broader circular band of olive green and rearward of this is a circular band of gray more or less tinged with brown. This gray color is due to the ends of white hyphae growing up from the mat beneath. The central and oldest area of the colony surface is usually dark gray to deep olive brown. The marginal contour is not fimbriate but uniformly circular. The reverse of the colony is jet black, and there is no diffusion of color into the substratum.

On a synthetic agar medium ($CaNo_3$, 1.22 gm.; KH_2PO_4, 2.44 gm.; $MgSo_4$, 3.69 gm.; and glucose, 20 gm. in 1 liter of distilled water) the color aspect was much the same as on potato agar, but the mat was not so dense and the diameter of the colony was smaller at the end of the same period. Fewer conidia were present on this medium.

[6] 200 gm. Irish potato, peeled, sliced thin in 1,000 c. c. distilled water. Autoclave 20 minutes at 15 pounds. Strain through cheesecloth, add 25 gm. chopped agar shreds and 20 gm. dextrose. Heat to boiling to melt agar. Adjust reaction to grass green to brom thymol blue. Autoclave. Readjust reaction. Filter through absorbent cotton, tube, autoclave.

The ability of the fungus to grow at different degrees of acidity was tested on potato-dextrose agar made as indicated above. The medium was adjusted to different hydrogen-ion concentrations by the addition of appropriate quantities of normal NaOH or HCl after the final sterilization. The cultures were made in triplicate in Petri dishes poured to a uniform depth of medium, uniformly inoculated, and incubated at 25° C. Colony diameters were recorded daily and taken as the measure of growth. On the alkaline side of neutrality growth occurred at the highest hydroxyl-ion concentration used, namely, that corresponding to a P_H of 9.6. At P_H 8.0, 8.5, 9.0, and 9.6 growth was slow in starting and at the two last-mentioned indexes proceeded at a markedly slower rate than on more acid media. At P_H 8.0 and 8.5, after the tardy start, growth continued at approximately the same rate as on less alkaline media. Growth was initiated most promptly, and continued at the highest rate in the range represented by P_H 3.6, 4.5, 5.5, and 6.9. No growth occurred at P_H 1.6. At P_H 3.6 the colonies reached a diameter of 75 mm. in 44 days and had the usual olive-green to olive-brown velvety surface composed of numerous short aerial hyphae. At P_H 2.6 growth was slow in starting, proceeded at a reduced rate, and produced colonies so abnormal in structure and appearance that they seemed to belong to an entirely different fungus. The colonies were small, having attained a diameter of only 13 mm. in 44 days, and had whitish or cream-colored pustulate or pimply surfaces with little or no suggestion of the usual dark-colored velvety aspect.

When the mycelium of these abnormal colonies is studied microscopically, it is seen, when young, to be composed of very short closely septate hyphae, and, when older, to consist of broken hyphal remnants whose cells have become greatly swollen. Apparently unable at the high acidity to function in normal-growth processes, the protoplasts within the original hyphal cells swell greatly, often bursting the mother-cell wall and each at the same time investing itself with a new thin hyaline wall. Remnants of the old mother-cell wall are frequently clearly visible clinging to the sides of the newly formed cells. (Fig. 9, G.) In swelling, the new cells become elliptical, oval, or spherical in shape, reach a diameter of 2 to 4 times that of normal hyphae, and often form septa which may or may not divide them into equal parts. The swelling is not accompanied by development of large vacuoles as if the energy of the cell were being exhausted in the mere process of enlargement, but the swollen cells are densely filled with protoplasm containing many coarser lumps or granules heaped in the center of each cell. These coarser lumps probably constitute a reserve of nutritive material. This phenomenon is suggestive both of chlamydospore formation in certain fungi and of the type of endogenous spore formation which occurs in the conidiophores of *Thielavia basicola* (*1*) and *Ceratostomella fimbriatum* (*6*). The process is not at all similar to budding such as occurs in germination of yeasts and certain other fungi. When these abnormally shaped and enlarged cells are placed in tap water, they germinate in the normal manner characteristic of conidia—i. e., by the formation of slender hyaline germ tubes (fig. 9, H), and when placed on less acid potato agar, they produce colonies of normal structure and appearance.

DISSEMINATION AND CONTROL

Laboratory tests have not yet been made to determine if the fungus can be isolated from seeds, nor has the disease yet been recognized on pods. However, field observations indicate that the disease is seed-borne. In mid-season observations made on variety tests, the disease has been observed on rows planted to certain varieties while adjacent rows of a different variety, although known to be susceptible, showed no disease until much later in the season. Had inoculation occurred as a result of a wind-borne spore shower from some unknown source, all susceptible kinds in a variety planting should become diseased at approximately the same time. As shown above, spores of the fungus may remain viable as long as 94 days when kept on dry leaves in the laboratory. It is not improbable that in the open field the fungus in leaf and stem lesions remains viable throughout the winter months. The fungus grows readily and sporulates abundantly on culture media and sterilized soy-bean stems. This would indicate that the fungus can grow saprophytically on dead soy-bean tissue and sporulate thereon in the following summer, thus creating a source of inoculum for the new crop. It is therefore recommended that all refuse from a diseased crop be plowed under in order to promote early decay as a means of reducing the amount of inoculum which may overwinter. In addition to this destruction of diseased material, the rotational practice should be such as to allow an interval of one full growing season between successive soy-bean crops on infested fields. In badly infected areas the earlier maturing, more resistant varieties of soy bean should be substituted for the late maturing and more susceptible varieties.

SUMMARY

The disease of soy bean herein described is designated "frog-eye leaf-spot."

The first American collection to which frog-eye may definitely be referred was made in Louisiana in August, 1925, and in North Carolina in September, 1925. It seems probable, however, that the same disease was actually seen by Moore in South Carolina in 1924.

Lesions occur chiefly on the foliage but have also been found on the stems.

The chief symptoms on leaves are rounded or angled necrotic lesions which are at first redish-brown but later have light brown to ash gray or even white centers, delimited by a narrow reddish-brown border. In old lesions the central tissues are often very thin and the border is often thicker than the surrounding undiseased tissue.

On stems the younger lesions have red centers bordered by a zone of black. Older lesions have pale smoke-gray centers surrounded by a narrow band of red and this in turn by an outer bordering band of black.

The disease is known to be present in at least five States, all of which are in the South. In North Carolina it is widely distributed over the piedmont and coastal plain areas.

Plants are frequently seen on which 25 per cent or more of the total leaf area is diseased. The disease is potentially capable of causing great reductions in yield of seed and quality of hay.

Greatest injury is done to late-maturing varieties such as Otootan and Biloxi. Early-maturing varieties such as Dixie, Manchu, and Virginia, although susceptible, escape serious injury.

The causal organism is readily isolated, grows well, and sporulates freely on many artificial substrata. The pathogene is described in detail in the body of this paper.

Conidia germinate readily in tap water, and a small proportion of them were still viable 94 days after collection, during which time they were kept on dry leaves in the laboratory.

Successful inoculations with conidia from pure cultures and the subsequent recovery of the same organism from lesions produced by such inoculations establish the pathogenic causal relation of the organism commonly associated with the disease.

The causal fungus is identified as *Cercospora diazu* Miura, first collected and described by Miura from south Manchuria in 1918. The fungus observed in North Carolina differs in certain minor morphological details from the description given by Miura, but it is held that these differences are not sufficiently pronounced nor yet sufficiently well fixed to justify the establishment of a new species for the organism present in America.

The fungus causes pathological changes in the host by means of some substance which acts in advance of the foremost hyphae. The host cells first show change in staining reaction. This is followed by protoplasmic disorganization and complete collapse of affected cells, only fragments of the cellulose walls remaining in the older portions of the lesions.

On potato-dextrose agar the causal fungus forms colonies characterized chiefly by a combination of prominent radial folds and less evident circular convolutions accompanied by concentrically arranged color bands of white, olive green, olive brown, and gray. Growth occurs over a range of acidity extending from P_H 2.6 to 9.6 or beyond. At P_H 2.6 growth is very slow and results in the formation of morphologically abnormal mycelium.

It is believed that the fungus may overwinter on diseased leaves and stems and on seed from diseased fields. The value of seed treatment has not yet been established. Control measures believed to be effective are (1) plowing under to promote early decay of infective material, (2) a rotation of two or more years, and (3) use of earlier and more resistant kinds of soy beans in place of late-maturing and more susceptible varieties.

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